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PARASITOLOGY

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Edinburgh: 100, PRINCES STREET
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PARASITOLOGY

A SUPPLEMENT TO THE
JOURNAL OF HYGIENE

EDITED BY
GEORGE H. F. NUTTALL, F.R.S.
Quick Professor of Biology in the University of Cambridge
AND
A. E. SHIPLEY, F.R.S.
Reader in Zoology in the University of Cambridge

Volume IV 1911

BRITISH MUSEUM
Z D
NATURAL HISTORY

Cambridge
at the University Press
1912

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS

Vol. 4, No. 4 December, 1911

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BRITISH MUSEUM Z D NATURAL HISTORY

THE LIFE-HISTORY OF *PLEISTOPHORA GIGANTEA*, THÉLOHAN (*GLUGEA GIGANTEA* THÉL.).

BY N. H. SWELLENGREBEL, PH.D.

(*From the Hygienic Institute, University of Amsterdam.*)

With Plates XVII and XVIII and 20 Diagrams.

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1. *Introduction.*

AMONG the Neosporidia the Microsporidia constitute a group distinguished from the allied Myxosporidia by the small size and simple structure of the spores. A full account of the literature on this subject is to be found in the excellent papers of Thélohan (1895), Stempell (1909), Schuberg (1910), etc.

According to Minchin's system (1903) the Microsporidia are divided into:

(1) *Polysporogenea*, the trophozoite (*i.e.* the vegetative stage of the life-cycle) produces many pansporoblasts, containing many spores.

(2) *Oligosporogenea*, the trophozoite produces only one pansporoblast containing four (Gurleya), eight (Thélohania), or many (Pleistophora) spores.

The parasite which I am about to describe has been found in a cutaneous tumour and in the mesenterium of *Crenilabrus melops.* Thélohan (1895) describes a parasite (*Glugea gigantea*) in the peritoneal cavity of the same fish; he does not mention any stages of the life-cycle, but only states that a polar filament was not to be found within the spores. It is difficult to ascertain the identity of Thélohan's parasite and mine, but this identity is probable considering the absence of a polar capsule in the spores of both parasites and the identity of the host. My parasite, however, is no *Glugea*, the trophozoite being directly transformed into a pansporoblast; consequently it is a *Pleistophora* and its name should be *Pleistophora gigantea.*

2. *Material and Methods.*

The different stages of the life-cycle of *P. gigantea* were found in a large tumour, situated on the ventral side of the head and the thorax of *Crenilabrus melops* (Pl. XVII, fig. 1), reaching from the posterior margin of the gills to the ventral fins. The tumour was caused by hypertrophy of the subcutaneous tissue; it did not show any sign of infiltrative growth: the gills and the organs of mouth and thorax were left quite unaffected. The tumour was so heavy as to deprive the fish of its hydrostatic equilibrium (*vide* Pl. XVII, fig. 1, representing the living fish).

Portions of the tumour were fixed in corrosive alcohol (after Schaudinn's prescription), washed in alcohol and iodine, hardened in absolute alcohol, mounted in paraffine, and cut into sections of 3–5 μ. The stains employed were hematoxylin (Ehrlich's, Delafield's, or Heidenhain's) or Giemsa's solution.

To demonstrate the polar filaments, living spores were treated with caustic soda or potash; sulphuric-, nitric-, and hydrochloric-acid, methyl alcohol, ether, iodine, and distilled water, but no filament was to be seen.

3. *Description of the Life-history of P. gigantea.*

The tumour already mentioned is composed of connective tissue and multinucleate giant cells. In this tissue are embedded cysts of different size containing spores and sporoblasts (Pl. XVIII, fig. 3). At times a large cyst is surrounded by a series of small cysts, suggesting that the spores of the former have caused a secondary infection of the surrounding tissue (Pl. XVIII, fig. 2). Occasionally the spores are disseminated throughout the connective tissue, without being enclosed within a cyst-wall (Pl. XVIII, fig. 4). This condition was called by Doflein (1909) "diffuse infiltration." The cysts may exceed 1·44 × 0·82 mm. in size, but some of them are much smaller with a diameter of 11–49 μ,

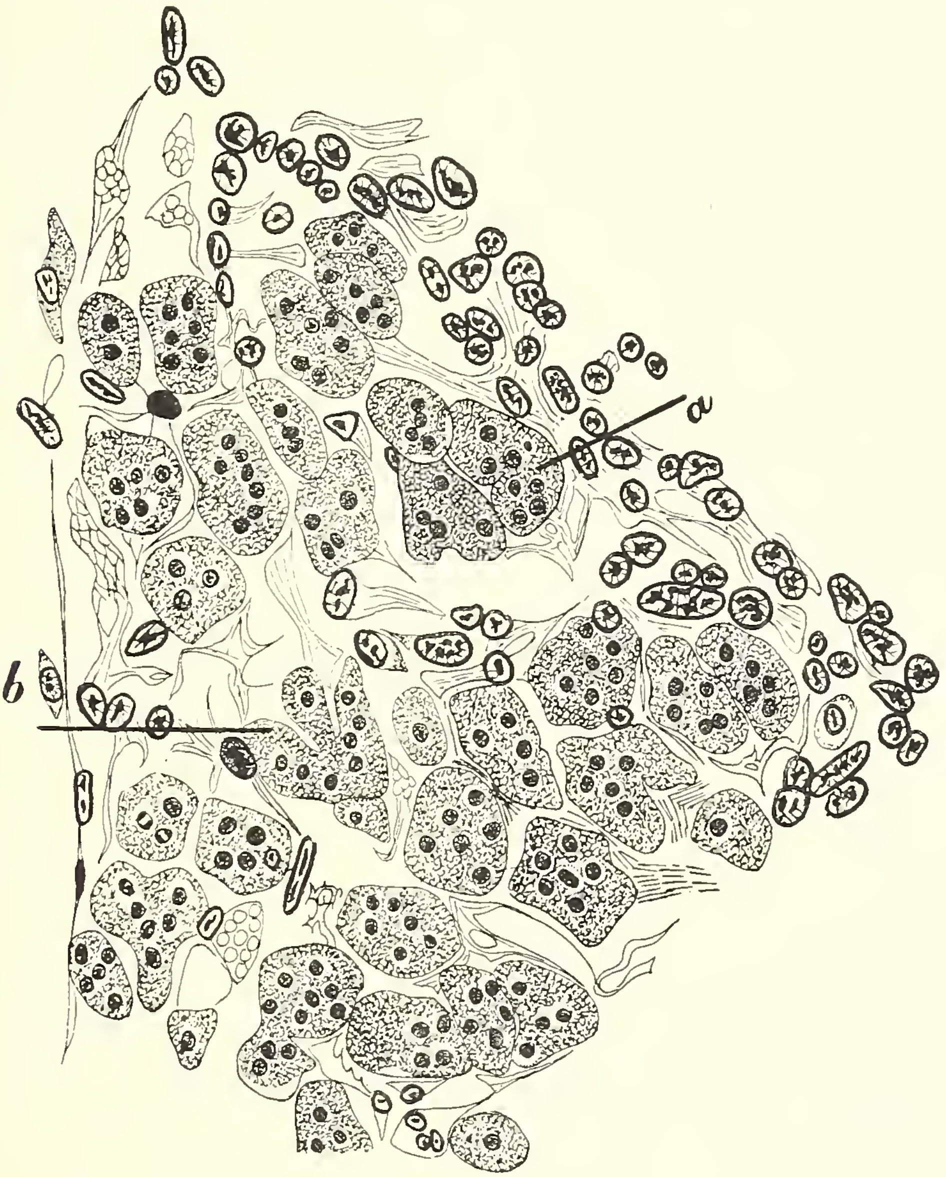

Diagram I. Trophozoites of *P. gigantea*. (Oil immersion 2 mm. ocul. No. 4.) Mono- and multinucleate individuals surrounded by connective tissue. *a*. Aggregation of 3 individuals. *b*. Stage of division.

and contain a relatively small number of spores. Between the cysts young individuals may be seen, even at a low magnification (Zeiss. Obj. 3, ocul. 2); they are free (Pl. XVIII, fig. 1 *a*) or surrounded by a thick capsule of connective tissue (Pl. XVIII, fig. 5 *a*).

The following observations were made with the aid of a high power of the microscope (Zeiss. Apochrom. oil immersion 2 mm., oculars 4 or 18).

(*a*) *Trophozoites.* The youngest developmental stages found are round or oval cells with one or more nuclei, 6·3–11·2 μ long and 4·6–9·8 μ broad (Diagram I; Pl. XVII, fig. 2). The protoplasm has an alveolar structure; the nuclei are very chromatophil and consequently they are deeply stained, which often checks the study of the internal structure. The nuclei are surrounded by a clear space. The protoplasm is enclosed within a thin membrane; this membrane is very distinct in individuals where the protoplasm shows signs of shrinkage (Diagram II, Fig. 1).

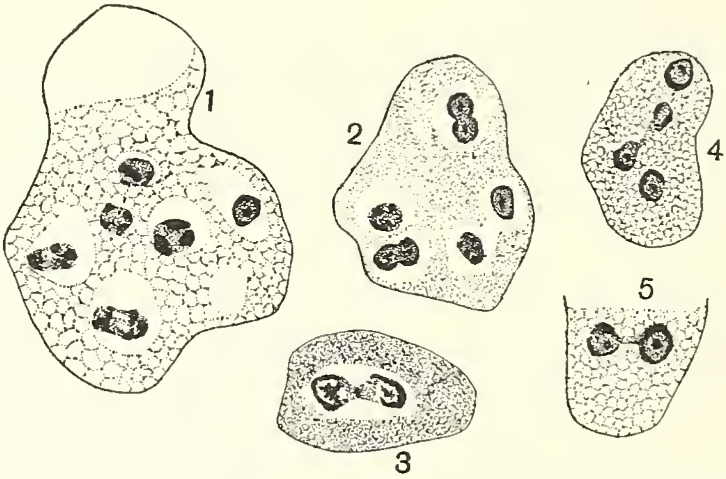

Diagram II. (Oil immersion 2 mm. ocul. 18.) Fig. 1. First stage of nuclear division. Fig. 2. Division of the karyosome. Fig. 3. Constriction of the elongated nucleus. Figs. 4, 5. Final stages of nuclear division.

The trophozoites contain one or more nuclei, and it is to be supposed that the mononucleate individuals are the youngest, considering that they are the smallest. Diagram I *b* suggests that the multinucleate individuals multiply by schizogony.

The structure of the nuclei of the trophozoites is a simple one (Diagram II), some chromatic granules are supported by an achromatic substratum. Sometimes a central chromatic granule is present which perhaps must be regarded as a karyosome. The nucleus which is about to divide becomes elongated and the chromatin is accumulated at the two poles (Diagram II, Fig. 1). At times the achromatic substance presents a distinct fibrillary structure; if a karyosomatic granule is present it divides also (Fig. 2). The nucleus becomes more and more elongated and shows a constriction in the middle; occasionally some chromatic substance may be observed in the neighbourhood of this constriction representing perhaps a rudimentary equatorial plate (Fig. 3). The final stage of the division is shown in Figs. 4 and 5, where a thin filament still unites the two daughter nuclei.

There is no close relation between nuclear and cellular division; a trophozoite may contain several nuclei before the beginning of cellular division.

Diagram I *a* shows three individuals placed in close contact; this becomes more apparent still in the trophozoites figured in Diagram III *bb*. Whereas Diagram III *a* shows a state of diffuse infiltration, the nuclei of the connective tissue being found between the parasites, we see that there is no place left for any nucleus between the trophozoites shown in Diagram III *bb*. These individuals form two compact masses which I will call "aggregations" in the course of this description. This process of aggregation is important because it is the beginning of encystment, as will be shown later on.

Sometimes the trophozoites are surrounded by a thick capsule of connective tissue, arranged in concentric layers (Diagrams IV, V and Pl. XVIII, fig. 5 *a*). Large nuclei are found situated so near the trophozoites that the latter might be regarded as intra-cellular parasites (Diagram V *a*, *b*). These encapsuled trophozoites show the same tendency to aggregation as the individuals of larger accumulations; the nuclei of the host's tissue disappear from the interstices between the trophozoites, the latter are closely joined together.

(*b*) *Formation of the pansporoblasts.* When a number of individuals combine to form an aggregation as do the individuals shown in Diagram III *bb*, they soon become surrounded by a membrane, thicker than the pelliculae of the trophozoites. I cannot exactly explain how this membrane is formed: perhaps it is constituted by the thickened pelliculae of the individuals situated at the periphery of the aggregation; if this supposition is right the membrane becomes very soon emancipated, as is shown in Diagram VII.

The number of individuals combining to encyst is very variable. In small cysts they are few in number (Diagram VII *b*), whereas in large cysts there are hundreds of them.

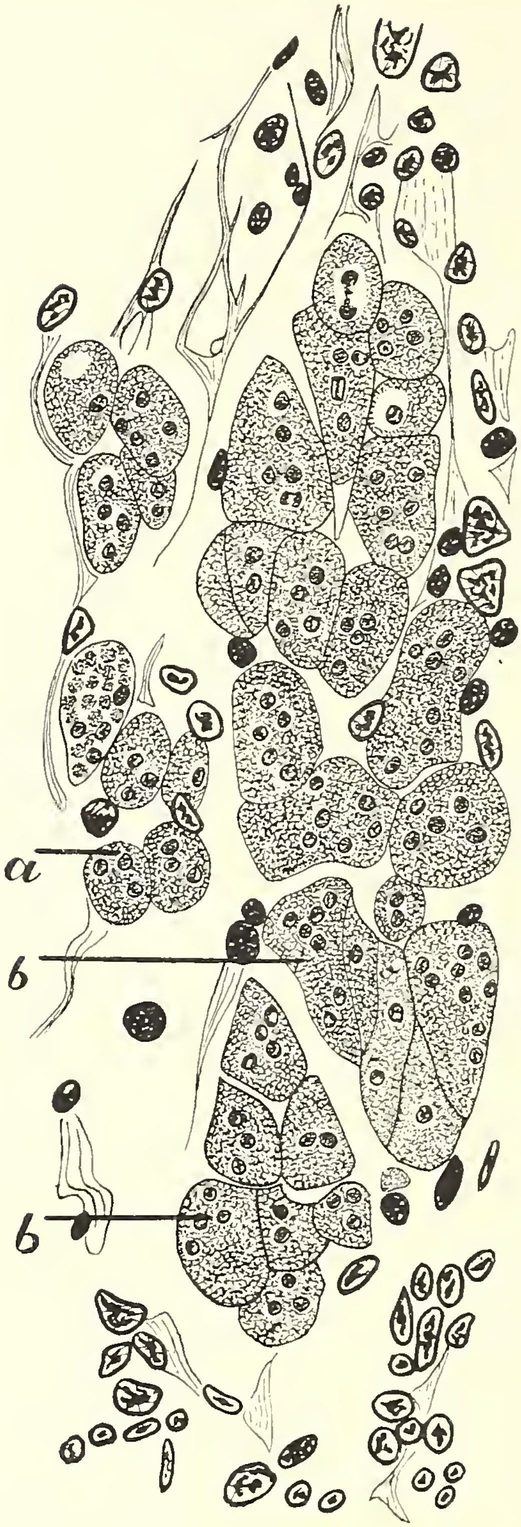

Diagram III. (Oil immersion 2 mm. ocul. 4.) *a.* Diffuse infiltration. *b, b.* Aggregation of 5 and 7 individuals.

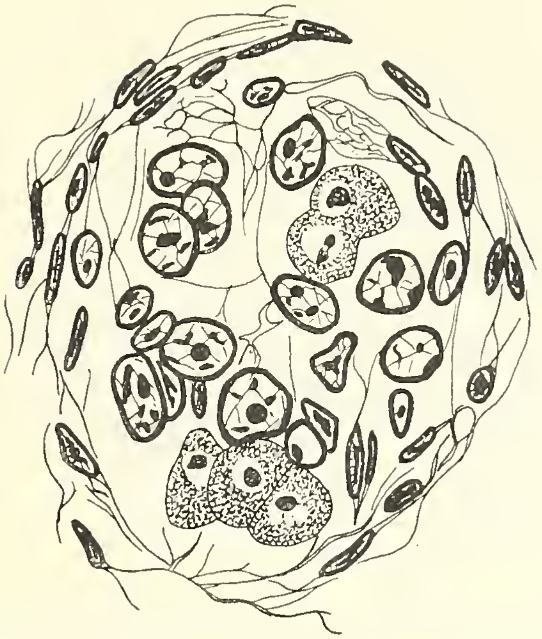

Diagram IV. (Magnification of Diagram I.) Four young trophozoites surrounded by a thick capsule of connective tissue.

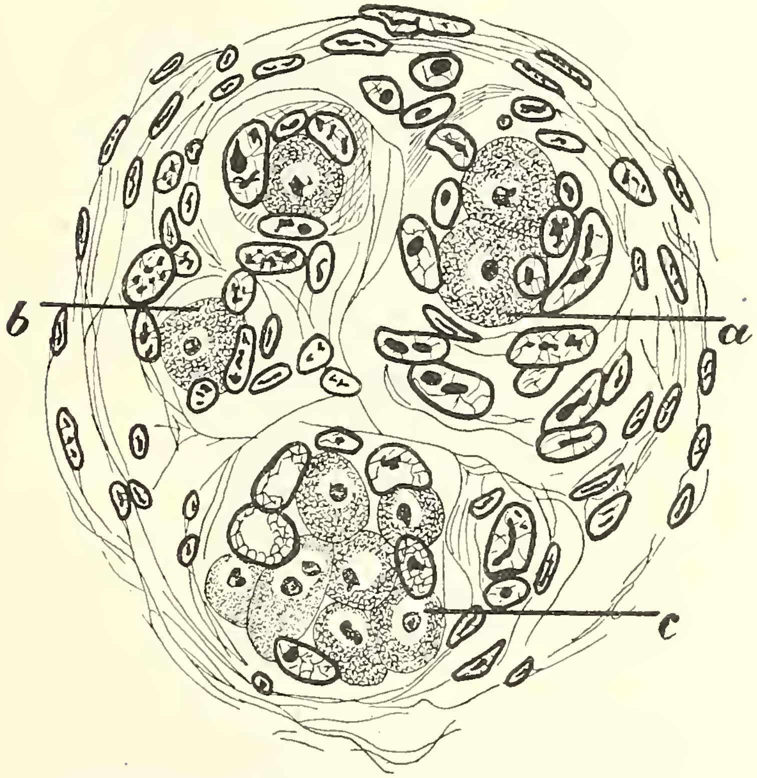

Diagram V. (Magnification of Diagram I.) Small aggregations of young individuals surrounded by connective tissue.

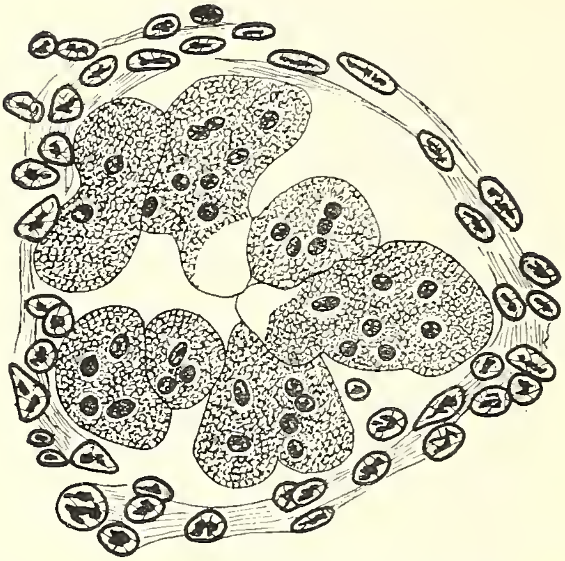

Diagram VI. (Magnification of Diagram I.) Aggregation of multinucleate trophozoites, surrounded by connective tissue. Cf. Plate XVIII, fig. 6.)

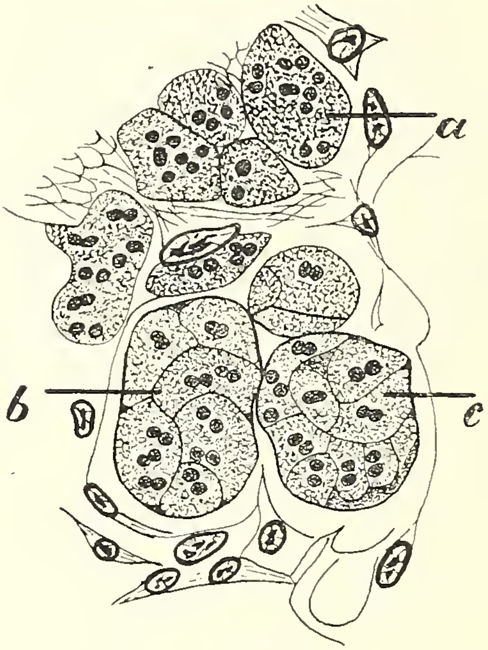

Diagram VII. (Magnification of Diagram I.) *a.* Trophozoites forming an aggregation. The nuclei are arranged in pairs. *b.* Complete aggregation. Nuclei arranged in pairs. *c.* Pansporoblast dividing to form small binucleate cells.

After the aggregation is completed we note that the nuclei of the trophozoites are no longer irregularly disseminated throughout the protoplasm but are arranged in pairs. This arrangement becomes gradually more apparent during the earlier stages of sporulation; it is caused by the daughter nuclei adhering to each other after nuclear division (Diagram IX). This division begins during and after encystment[1].

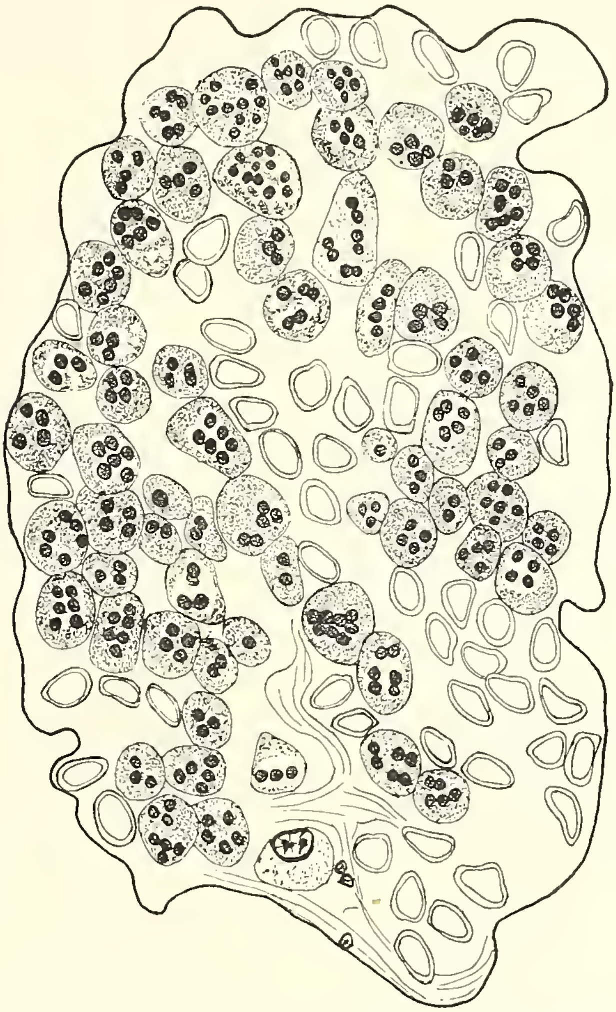

Diagram VIII. (Magnification of Diagram I.) Large cyst containing pansporoblasts with pairs of nuclei and spores. Only the outlines of the spores are drawn. In the inferior part of the cyst some elements of connective tissue are to be seen.

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[1] It must be remembered that the diagrams only show small cysts (except Diagram XI); this is for convenience sake because drawings of large cysts would take too much place. There is, however, no reason to suppose that sporulation in large cysts is performed in a way different from that followed in small cysts.

After the encystment the trophozoites become rounded and are no longer in so close contact with each other as they were before (Diagram VII *b*, *c*, Diagram VIII). Consequently, the individuals do no longer fill the whole lumen of the cyst; between them larger and smaller interstices are left.

This change is caused by the reduction of the protoplasmatic substance in the trophozoites, whereas the amount of nuclear substance is increased. This becomes evident when comparing some of the trophozoites figured in Diagram VIII containing 5–11 nuclei, with the larger trophozoites shown in Diagram III, which contain an equal or smaller number of nuclei.

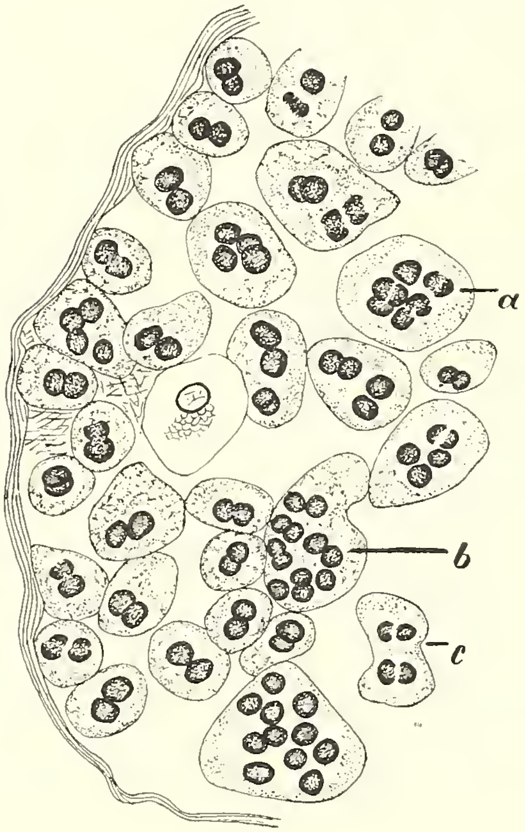


Diagram IX.

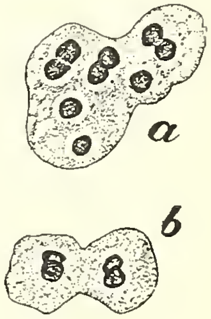


Diagram X.

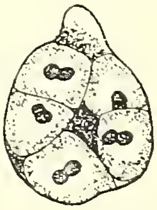


Diagram XI.

Diagram IX. (Magnification of Diagram II.) Part of the cyst shown in Diagram VIII under a higher power. *a*. Pansporoblast with nuclear division leading to the formation of pairs of nuclei. *b*. Pansporoblast with nuclei arranged in pairs. *c*. Dividing pansporoblast with two pairs of nuclei.

Diagram X. (Magnification of Diagram III.) *a*. Pansporoblast producing a sporoblast by unequal division. *b*. Pansporoblast producing sporoblast by equal division.

Diagram XI. (Magnification of Diagram I.) Small cysts with few sporoblasts.

These individuals, rich in nuclear material, will produce the sporoblasts and consequently are to be considered as pansporoblasts. Their protoplasm does not stain so deeply as that of the trophozoites but it has a similar alveolar structure. They have a diameter of 4·9–6·3 μ.

The formation of the sporoblasts is represented in Diagrams IX and X.

(*c*) *Formation of the sporoblasts.* The sporoblasts do not arise by a schizogonic division of the pansporoblasts as is commonly observed among microsporidia; it seems that they are formed by unequal (Diagram X *a*) or binary (Diagrams IX *c*, X *b*) fission, each sporoblast receiving one pair of nuclei.

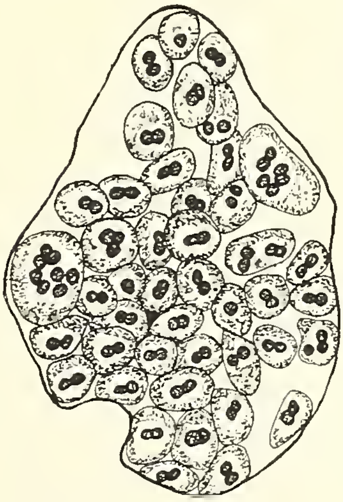


Diagram XII.

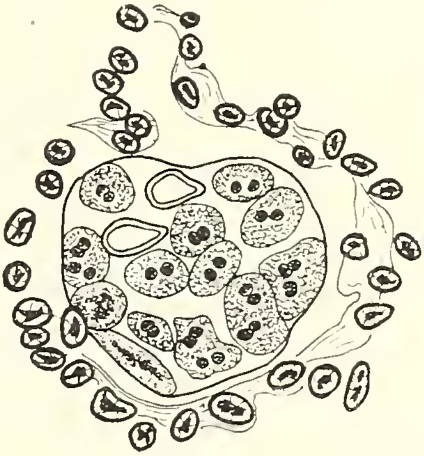


Diagram XIII.

Diagram XII. (Magnification of Diagram I.) Large cysts with sporoblasts and four pansporoblasts.

Diagram XIII. (Magnification of Diagram I.) Small cysts with sporoblasts, pansporoblasts, and spores (only the outlines of the spores are indicated).

The nuclear division producing the pairs of nuclei is a simple fragmentation (Diagram IX *a*). The nuclei of the pansporoblasts do not show any distinct internal structure, the periphery is more chromatic than the centre. The dividing nucleus becomes elongated; the two poles are covered by the chromatic substance; in the middle no chromatine is left. Finally, the nucleus is divided by an equatorial fissure.

Considering that elements of the host's tissue were to be found among the trophozoites, it is not surprising that nuclei and fibres of connective tissue are occasionally observed within the cysts (Diagrams VIII and IX).

It is impossible to determine the exact number of sporoblasts produced by a single pansporoblast, because the sporoblasts do not remain attached to each other after division as is the case in other microsporidia, but judging by the number of the pairs of nuclei contained in a pansporoblast this number must be about 3–6. The number of sporoblasts contained in a cyst is of course dependent on the size of the latter, which is very variable (cf. Diagrams XI, XII).

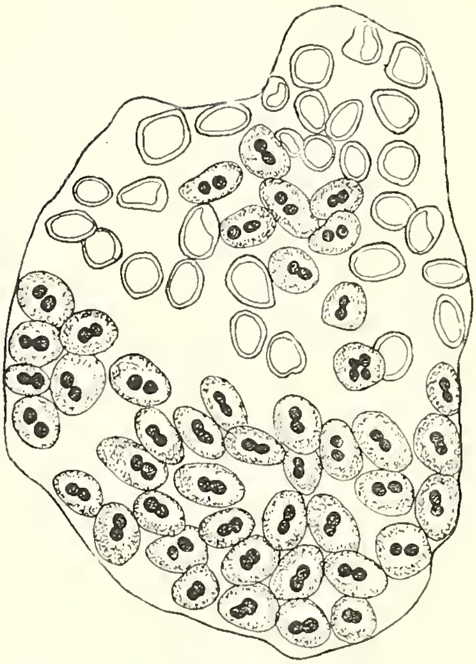


Diagram XIV.

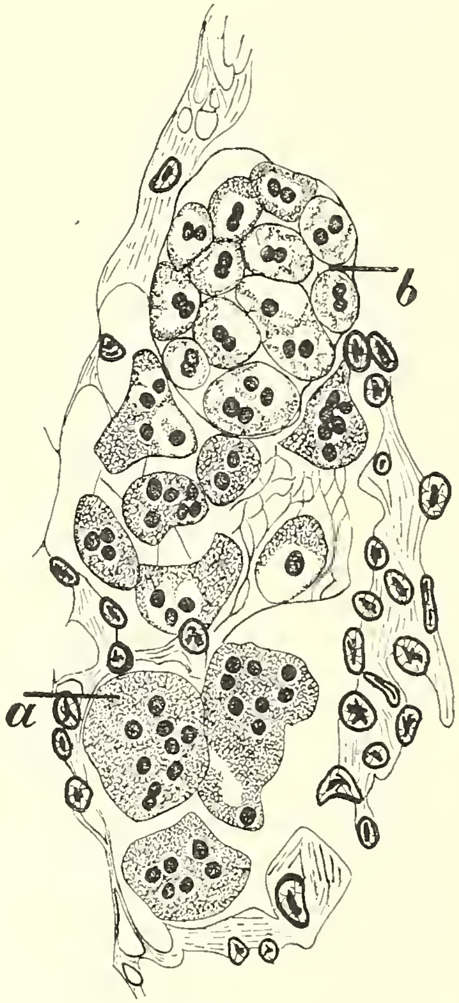


Diagram XV.

Diagram XIV. (Magnification of Diagram I.) Large cysts with sporoblasts and spores (only the outlines of the spores are indicated).

Diagram XV. (Magnification of Diagram I.) Cyst (*b*) with sporoblasts and pansporoblasts, surrounded by connective tissue containing three trophozoites (*a*).

At first some pansporoblasts are still to be seen among the sporoblasts (Diagrams XII, XV), gradually they disappear and only sporoblasts are left, which finally become spores. Consequently in Diagrams XIII and XIV the outlines of the spores are likewise to be seen.

The sporoblasts contain two nuclei, similar in structure to those of the pansporoblasts. These nuclei are often situated in so close contact that they seem to constitute one large nucleus (Plate XVII, fig. 3). The protoplasm of the sporoblasts does not stain well; round the nuclei a clear space is left and in the protoplasm large vacuoles are to be seen (Diagram XVII). When the sporoblasts are just formed these vacuoles are not yet present.

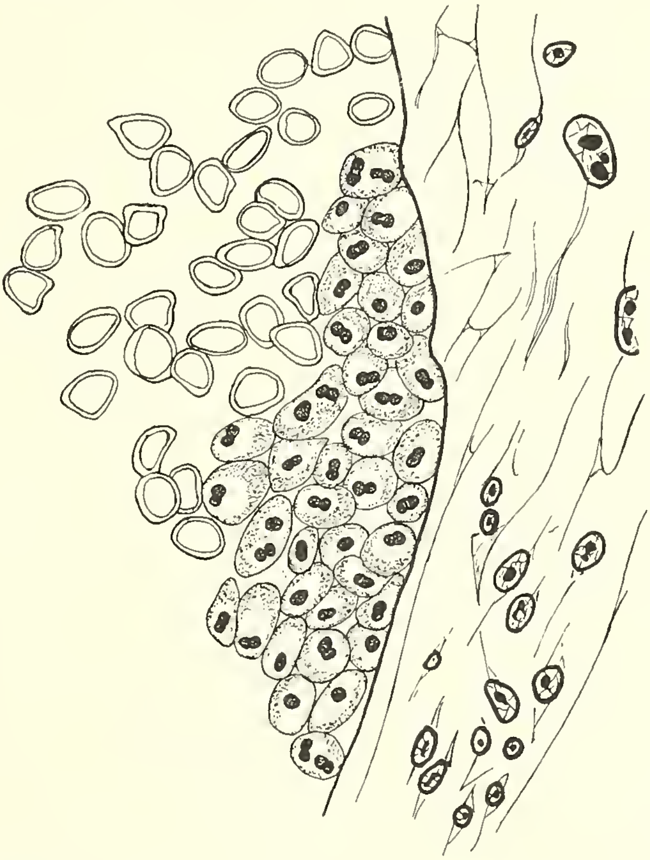


Diagram XVI. (Magnification of Diagram I.) A portion of the surface of a large cyst with sporoblast and spores (only the outlines of the spores are indicated).

These intermediate stages, leading to the formation of the sporoblasts, are to be observed only in the smaller cysts, the large ones only contain sporoblasts and spores (Diagram XVI).

The sporoblasts are covered by a thin membrane. During their formation there has been an increase of protoplasmatic substance, consequently they are placed so closely together that each sporoblast takes a more or less distinct angular shape. The membrane of each sporoblast sticks readily to the membranes of the surrounding sporoblasts, consequently all the sporoblasts, and even the neighbouring spores, hold together, forming a pseudo-tissue, resembling somewhat the parenchymatous tissue of a plant. The sporoblasts have a diameter of 3·5–4·9 μ.

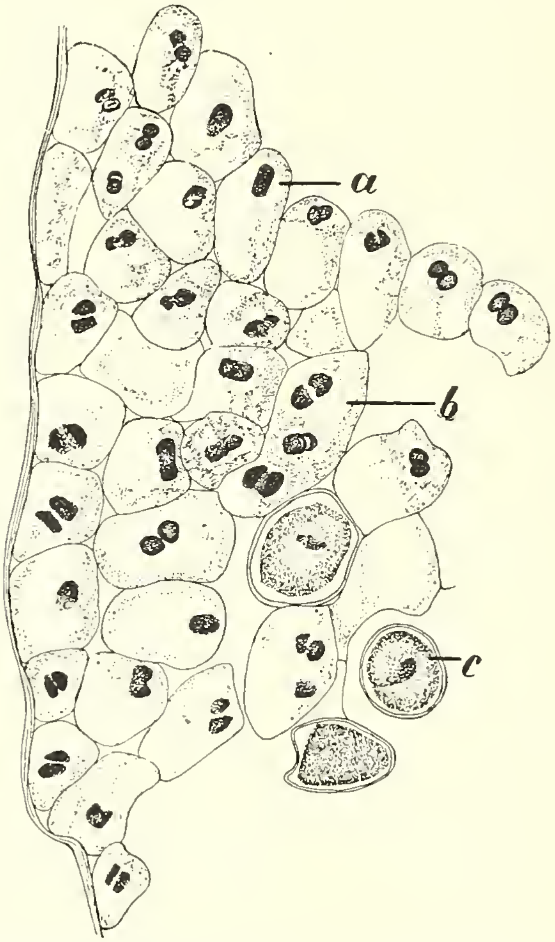


Diagram XVII. Representing the same cyst as that shown in Diagram XVI, but at a higher magnification (of Diagram II). *a*. sporoblasts. *b*. pansporoblast with three pairs of nuclei. *c*. spores.

(*d*) *Formation of the spores.* When a sporoblast is transformed into a spore, the membrane becomes much thicker, the protoplasm loses its vacuoles and is deeply stained. In living spores the protoplasm fills the whole lumen within the spore-wall, but in fixed and stained preparations this is not the case, because of the contraction of the protoplasm. The nuclei are not so pycnotic as those of the sporoblasts. At first the walls of the spores stick to the surrounding sporoblasts (Diagrams XVII and XVIII), afterwards they become free but still adhere to each other in larger or smaller clusters, because of the sticky condition of the surface of the spore-wall.

The young spores are distinctly binucleate, the two nuclei touching each other (Diagram XVIII, Fig. 2 *a*, *c*). In older spores only one large nucleus is to be seen (Fig. 2 *d*), consequently it may be supposed that this single nucleus does arise by the fusion of the two nuclei. It is impossible to explain in another way how the sporoblasts, all of them being binucleate, could produce mononucleate spores.

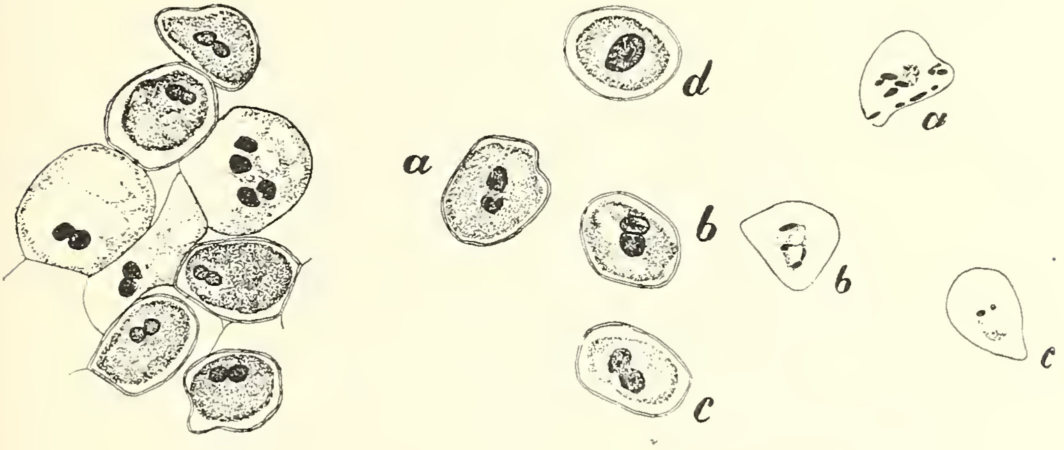


Fig. 1. Fig. 2. Fig. 3.

Diagram XVIII. (Magnification of Diagram II.) Fig. 1. A pansporoblast, sporoblasts, and spores adhering to each other. The lines connected with the walls of the spores and sporoblasts represent portions of the membranes of other sporoblasts which have been cut away by the microtome. Fig. 2. *a–c*. Binucleate spores, Giemsa's stain. *d*. Mononucleate spore, Giemsa's stain. Fig. 3. Spores showing volutin granules. *a*. Diffuse dissemination of the volutin. *b*. Intranuclear volutin.

Occasionally the spores contain chromatophil granules which are not decolorised by sulphuric acid (1%) or Gram's iodine (after staining with methylene blue). Consequently these granules consist of volutin, a substance also found by Schuberg (1910) in the spores of *Pleistophora longifilis*. The volutin granules are either disseminated throughout the spore (Diagram XVIII, Fig. 3 *a*) or they are to be found in the neighbourhood of the nucleus.

No polar capsule was to be seen in the spores and no polar filament could be demonstrated by the ordinary methods (see Material and Methods). The spores are 4·4–5·6 μ long and 3·4–4·6 μ broad.

(*e*) *Diffuse infiltration.* It has been already stated that the spores are not always to be found situated within cysts; at times they are spread throughout the connective tissue (Plate XVIII, fig. 4; Diagram XIX). These spores are probably produced by individuals which do not aggregate and encyst but I have been unable to ascertain if this is the case, never having observed non-encysted individuals in the act of sporulation.

(*f*) *Reaction of the host's tissue against the parasitic invasion.* I have already described how the trophozoites are sometimes surrounded by a thick capsule of connective tissue (Diagrams IV, V, VI).

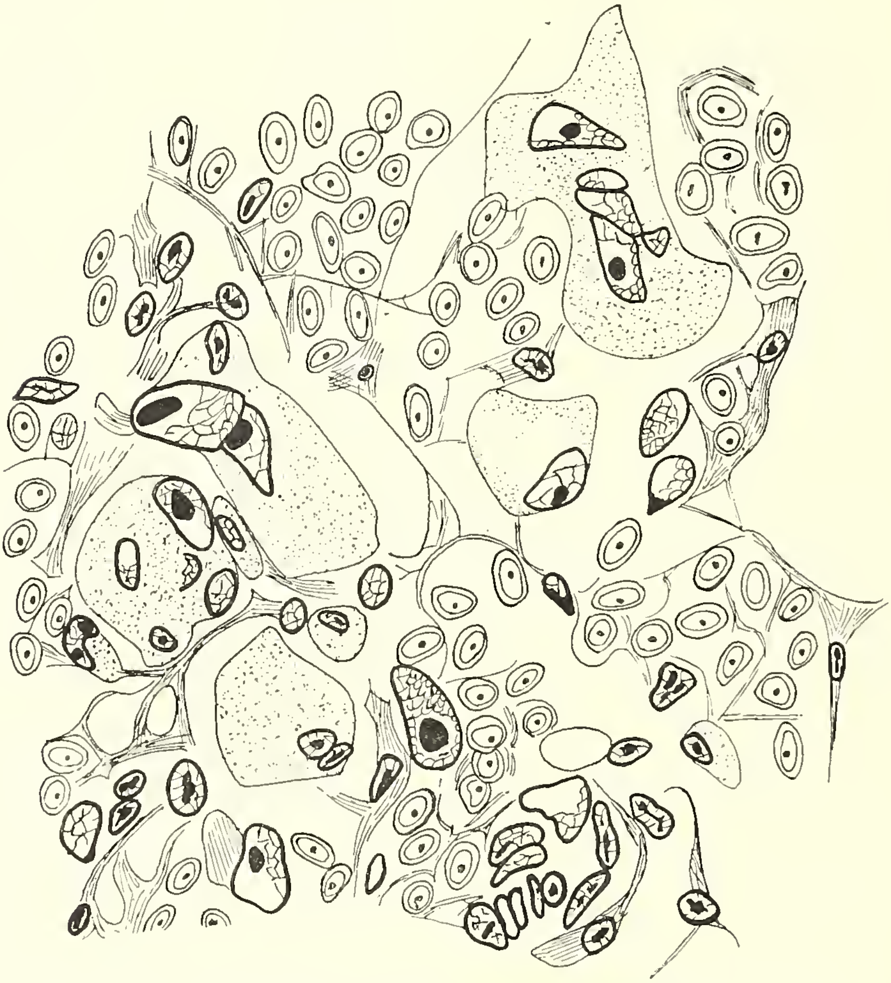


Diagram XIX. (Magnification of Diagram I.) Diffuse infiltration of spores.

This hypertrophy of the connective tissue is probably to be regarded as a protective reaction against the invasion of the trophozoites. It is only to be seen where few parasites are present: around large accumulations of trophozoites (Diagrams I, III) no such hypertrophy was observed.

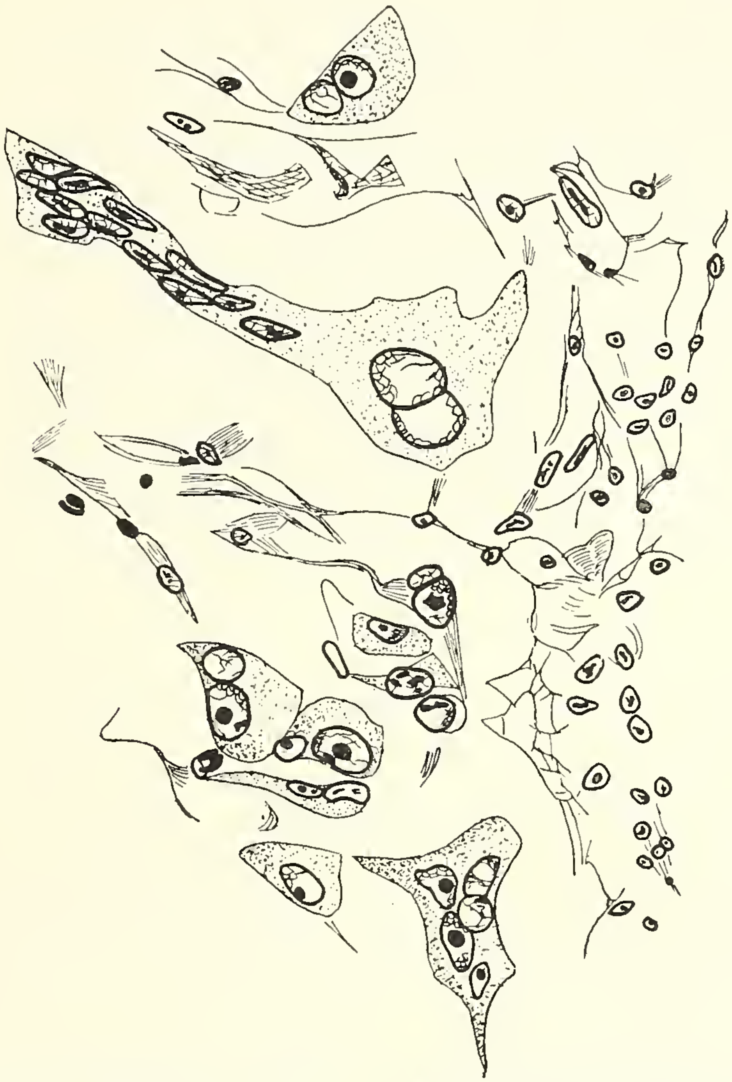


Diagram XX. (Magnification of Diagram I.) Multinucleate giant-cells in an area of diffuse infiltration.

In areas of diffuse infiltration large multinucleate giant-cells are to be found, but they are generally absent where the spores are formed within cysts. These giant-cells may contain 2–15 nuclei and attain dimensions of 28–43 μ (Diagrams XIX, XX).

Summary.

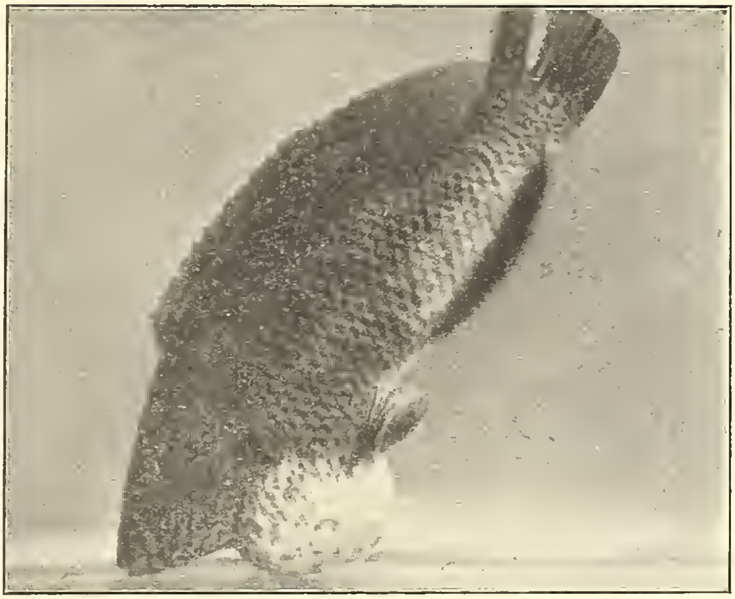
The parasite which I have here described has a life-history containing two distinct stages: a vegetative stage and a sporogonic stage. During the vegetative period multinucleate individuals divide by irregular schizogony producing young uni- or paucinucleate individuals. During the sporogonic period, a number of trophozoites unite to form an aggregation which becomes encysted. In the encysted individuals (the pansporoblasts) there is an increase of nuclear substance and a reduction of plasmatic substance. The nuclei become arranged in pairs and by successive unequal divisions the pansporoblasts produce the sporoblasts which contain a pair of nuclei each. During the formation of the sporoblasts there is an increase of-protoplasmatic substance. The sporoblasts become spores by the thickening of their pelliculae.

At first the spores contain two nuclei, finally they become mononucleate. This must be explained by the fusion of the two nuclei.

This nuclear fusion is probably to be interpreted as a paedogamous autogamy (in accordance with the nomenclature of Hartmann, 1909).

The systematic position of this parasite.

According to Minchin's classification, already mentioned, it is to be ranged among the Oligosporogenea because the trophozoite is directly converted into a pansporoblast. The number of spores produced by a single pansporoblast is not constant, consequently the parasite might be considered to belong to the genus *Pleistophora*, but in *P. periplanetae* (Perrin, 1906), *P. longifilis* (Schuberg, 1910), etc. we note that the spores are formed within the pelliculae of the pansporoblast and not in the way described here. Moreover, the structure of the spores of these *Pleistophorae* is very different from that of the parasite of *Crenilabrus melops*; the aggregation and the united encystment of several individuals as an introduction to sporogony, are never mentioned in the description of the genus *Pleistophora*. Consequently it is with some restriction that I range this parasite among the members of this genus, I do so only to avoid the creation of new and perhaps unnecessary genera.



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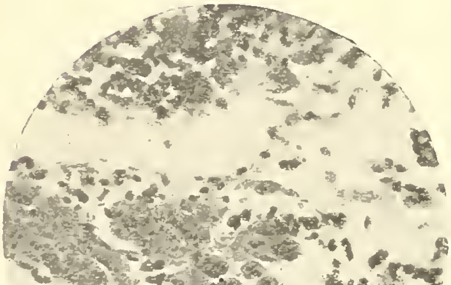

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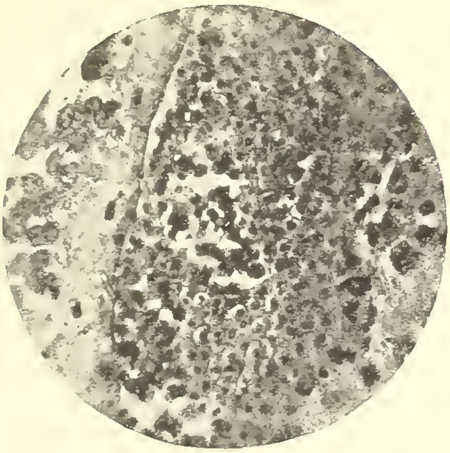

Fig. 3.

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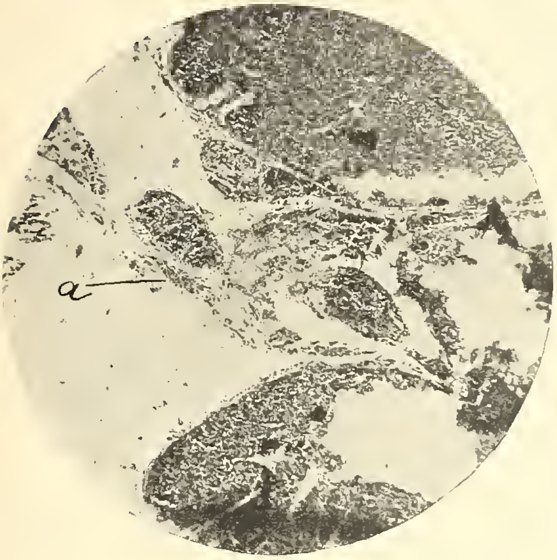

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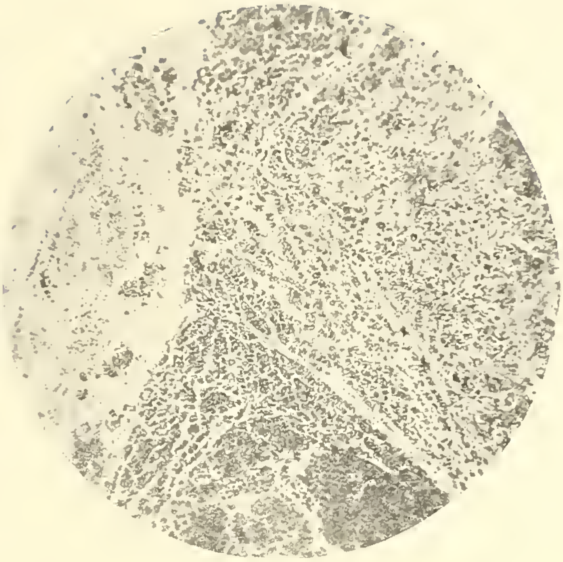

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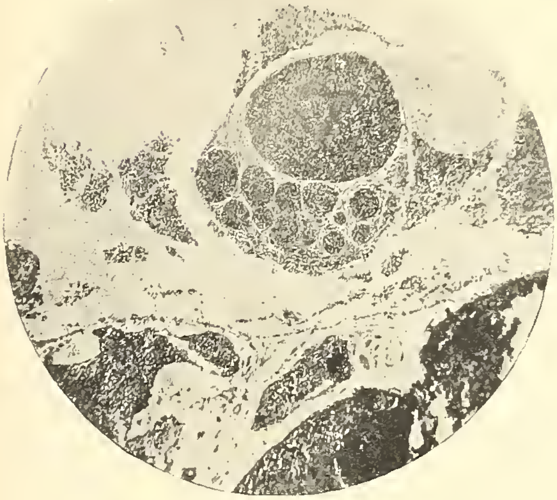

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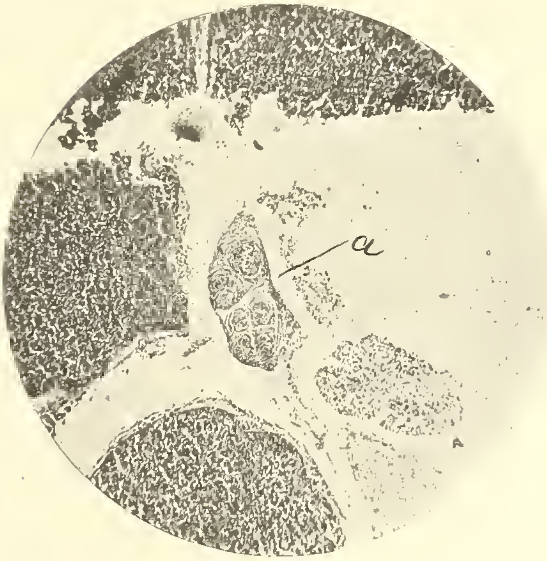

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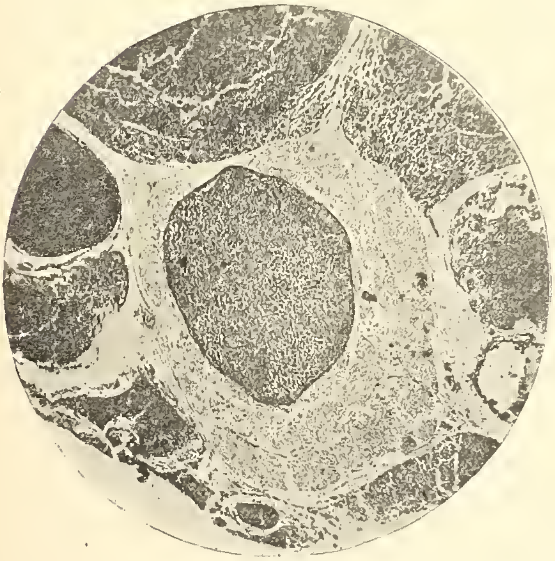

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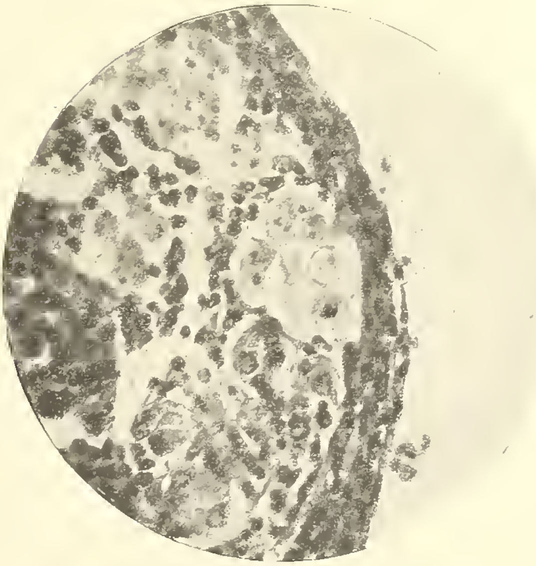

Fig. 6.

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NATURAL HISTORY

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DESCRIPTION OF PLATES XVII AND XVIII.

PLATE XVII.

Fig. 1. Living specimen of *Crenilabrus melops* with tumour caused by *Pleistophora gigantea.*

Fig. 2. Trophozoites of *P. gigantea* surrounded by connective tissue. (Zeiss oil immersion 2 mm. ocul. 2.)

Fig. 3. Portion of a large cyst of *P. gigantea*, containing sporoblasts. (Zeiss oil immersion 2 mm. ocul. 4.)

PLATE XVIII.

Fig. 1. Cysts of *P. gigantea. a.* Group of trophozoites between the cysts. Zeiss obj. 3 ocul. 2.

Fig. 2. Large cyst of *P. gigantea* surrounded by smaller cysts. (Magnification of fig. 1.)

Fig. 3. Large cyst of *P. gigantea.* (Magnification of fig. 1.)

Fig. 4. Diffuse infiltration of spores of *P. gigantea.* (Magnification of fig. 1.)

Fig. 5. Cysts of *P. gigantea. a.* Capsules of connective tissue surrounding trophozoites. (Magnification of fig. 1.)

Fig. 6. Trophozoites tightly encapsuled by connective tissue. (Zeiss oil immersion 2 mm. ocul. 4.)

TETRARHYNCHUS ERINACEUS VAN BENEDEN.
I. STRUCTURE OF LARVA AND ADULT WORM.

By JAMES JOHNSTONE, B.Sc.
(*Fisheries Laboratory, University of Liverpool.*)

With Plates XIX—XXIV and 9 Text-figures.

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I. *Introduction.*

My attention was first directed to this Cestode in 1906 when I was asked to examine several specimens of edible fish which had been exposed for sale in the public markets and had been condemned as human food on account of the presence of obvious, intrusive bodies in the flesh. In three of these cases the muscles of the flesh of the fishes —a Halibut, a Megrim and a Hake—contained cysts which enclosed the larvae of a Tetrarhynchid, and on examination the latter was identified as *T. erinaceus* from the peculiar nature of the armature of the proboscides. In another case the wall of the stomach of a Halibut was also infected with the same larvae. Acting on an obsolete principle of Border Law the Fish Inspectors promptly condemned the fishes and then caused them to be examined. I do not think there was any valid reason for the condemnation of the articles of food, for I can find no reference in the literature to the presence of Tetrarhynchids in the human alimentary canal, and there does not appear to be any question of a possible communication of a parasite by the consumption, as food, of such infected flesh. Further the number of larvae present was far too few to lead to any emaciation of the fish[1], or to the likelihood of the production of toxic substances in the flesh of the animal. Nevertheless the Inspectors probably acted in the interests of the public health in condemning such articles of food as contained obvious cyst-like structures in the flesh, as to the precise nature of which they were ignorant, since there is always the possibility that these bodies might be detrimental to the health of those eating them. Further the presence of the cysts was very obvious and rather unpleasant to the eye, and might easily have prejudiced a customer against a particular vendor, or fish. However this may be my attention was thus directed to the Cestode and it was found that it was widely distributed in Irish Sea fishes. On searching the literature I also found that though there are many mentions of the occurrence of the worm there is no detailed description of its structure; and it seemed that an adequate investigation of this interesting form might be of considerable importance. The present paper is therefore the first instalment of such a study.

II. *Distribution and life-history.*

Tetrarhynchus erinaceus appears to have first been described by P. J. van Beneden in 1850 from the Angler-Fish (*Lophius piscatorius*) and the Cod (*Gadus callarias*) in which fishes it was present as encysted larvae; and from various species of Rays where it was present in the sexually mature phase (van Beneden 1858). The same author has also given a general account of the development of the mature worm from the encysted scolex, and a general account of the anatomy of the proglottis, which although lacking in detail is very accurate so far as it goes. Pintner (1880 and 1893) has described the general anatomy of several species of *Tetrarhynchus* (but not *T. erinaceus*); and Vaullegeard (1889) has described, without much detail, the organs of the Cestode in question. Vaullegeard's paper is a general account of the whole genus so far as it was known, and it includes a very exhaustive bibliography of the memoirs written with respect to Tetrarhynchids. I refer the reader to this paper rather than attempt to give a detailed list of the literature relative to *T. erinaceus.* Linton (1891) has also described an apparently new species under the name of *Rhynchobothrium imparispine,* but Vaullegeard refers this species to *T. erinaceus.* I am not sure that the two species are identical but in the absence of a more detailed description than that given by Linton it is impossible to be sure of this. There are also other references in the literature to the occurrence of *T. erinaceus,* but with the exceptions of van Beneden's first accounts and Vaullegeard's memoir, neither of which deals in detail with the anatomy of the worm, there are no good accounts of the species.

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[1] Such an emaciation as I have observed to result from the infection of a fish by the larvae of a Trematode worm; see Johnstone (1905); Nicoll (1909); and Lebour (1907).

I began in 1907 to work on van Beneden's species but have always found difficulty in obtaining sufficient material. The mature worm occurs in the large intestine of various species of Skate and Ray: *Raia clavata, R. maculata, R. batis, R. circularis* and *R. microcellata,* all obtained from the fishing grounds in the Irish Sea. It is always attached to the spiral valve at the anterior end of the large intestine, though free proglottides may be found quite near to the anus. The gut of these fishes is best examined for tapeworms and flukes by dissecting it out, and slitting it up with a pair of scissors so that every fold of the spiral valve is cut through. The gut is then placed in a photographer's developing dish containing water in which stands a large "crystallising dish" filled with water; and then fold after fold of the spiral valve is lifted over the edge of the crystallising dish and scraped with the back of a scalpel. In this way one can hardly fail to see any Trematodes or Cestodes that may be present. Any tapeworms found are best preserved as entire objects by killing them in fresh water and then fixing in 3 per cent. formalin: this method is not good for sections of the worm, but one obtains beautifully relaxed specimens with bothridia, etc. fully expanded, and with the tissues very transparent so that good whole cleared preparations can be obtained.

Fixation for minute histological study is best effected by the use of Zenker's fluid; other mercuric chloride fixatives do not appear to give nearly such good results. Fixation in vom Rath's fluid was also tried, followed by staining in iron haematoxylin but the stain is not a general one, although good for some details.

The sexually mature worm is comparatively rare in Irish Sea fishes, and as a general rule I have had to dissect from ten to twenty fishes in order to obtain one tapeworm. This paucity of material has rendered the investigation, so far, an incomplete one. Obviously the details of the life-history, such as the development of the Oncosphere and Plerocercoid stages, and the precise means of infection of the larval and final hosts, can only be properly studied by feeding experiments and for this purpose abundant material has not yet been available. Lack of material has also prevented some much needed work on the finer details of the histology of the central nervous system, since in investigations of this nature many experiments have to be made before satisfactory methods of fixation and staining can be evolved.

The Plerocercoid larvae of Tetrarhynchids, chiefly *T. erinaceus*, are among the most abundant of Helminth parasites of some Irish Sea fishes. I have observed them from *Trigla gurnardus* and *T. hirundo*, *Gadus callarias*, *G. merlangus*, *G. aeglefinus*, *Merluccius vulgaris*, *Scomber scomber*, *Hippoglossus vulgaris*, *Pleuronectes platessa*, and *Arnoglossus laterna*, and there are no doubt other larval hosts. The cysts containing the larvae are generally abundant, and it is rare, in my experience, for Whiting and Gurnards to be free from infection. In a Whiting stomach in the Fisheries Laboratory at Piel, Barrow-in-Furness, there are over 130 encysted larvae in the wall; and in a Halibut stomach examined some time ago there were nearly as many. The larvae may be found in various parts of the tissues of the host but they are very generally found on the mesenteries, or are present on the peritoneum covering the stomach and intestine: I have never seen them in or on the liver[1], or free in the body cavity. They are present exceptionally in the muscles: this was the case in several fishes—Hake and Halibut—examined by me. In these fishes the flesh round the vertebral column contained numbers of oval or cylindrical cysts which included Plerocercoid larvae. Little cavities had formed among the muscle bundles by the intrusive organisms. Many of these larvae were degenerate and the cysts contained only calcareous corpuscles with some obscure connective tissue. This may also be the case with the cysts found on the mesenteries or peritoneum.

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[1] Since writing the above I have obtained four specimens of the peculiar Tetrarhynchid *Coenomorphus linguatula* (van Beneden) from the Cod-Fish (*Gadus vireus*). It was attached to the liver of the host.

I have not, in the meantime; any observations to record with reference to the life-history of *Tetrarhynchus erinaceus.* The scheme usually adopted is that given by Braun (1894–1900, p. 1609) and this is purely conjectural. The identification of *Trigla* as the larval host is based solely on the recognition of the scolex of the Plerocercoid larva as that of *T. erinaceus.* As to this identification there is very much doubt: there is no doubt, of course, that the larva inhabiting the cysts found in *Trigla* is really that of *T. erinaceus,* but it is far from being certain that the Rays are infected by eating species of Trigla and other Teleost fishes containing these Plerocercoid larvae. In order to prove that *Trigla,* or *Gadus merlangus,* or any other of the Teleostean fishes mentioned above are really larval hosts in the proper sense we should have to show (1) that they are themselves infected by eating proglottides voided by the Ray, or that they eat some invertebrate, which is infected by eating the proglottides; (2) and that the Ray becomes infected by eating *Trigla* or any other Teleost fish containing the Plerocercoids. Now there is no evidence whatever that these things happen, and no feeding experiments have been made with the object of establishing the life-history. The eggs of the worm develop within the uterus but I have never seen the six-hooked larva in this situation. Possibly further development may occur after the dehiscence of the mature proglottis from the parent strobila, while the former is still within the intestine of the Ray, or even while it is lying on the sea bottom after having been passed by the fish. Now the free proglottis is only about 0·5 cm. in length and it is very unlikely that such a small object should be eaten by fish like Gurnards, Whiting, etc., which habitually feed on much larger organisms. It is, of course, quite possible that even such small objects may be eaten by the fishes accidentally, but the infection of the so-called larval hosts is so general that we can hardly suppose the process to be a matter of accident. I think it is far more likely that the ripe proglottides are eaten by some small invertebrate, a crustacean or mollusc or small fish, and that the latter is then eaten by the so-called larval host.

It is highly unlikely that the Ray is infected by eating such fishes as Gurnards or Whiting containing the Plerocercoid larva. Ray usually eat crustacea and quite small fish, and the assumption that they receive the tapeworm from a Teleost fish is not supported by an examination of their food. It is more probable then, that the adult host is infected by eating some invertebrate which acts as the true larval host and in which some larval form of *Tetrarhynchus* lives. What then is the place of the Plerocercoid worms inhabiting the Teleosts in the life-history? We notice that very many fishes may act as this host, and that in these animals we only find two conditions of the larva: (1) the Plerocercoids containing fully developed scolices, and (2) degenerate forms of larvae. The Teleosts are final, not intermediate hosts, and the larvae *Tetrarhynchi* inhabiting their tissues degenerate and carry the life-history no further.

This is the conclusion come to by Southwell (1911) with regard to the Ceylon *Tetrarhynchus unionifactor*. The conjectural life-history of this species is as follows:—(1) a larval host (a mollusc) containing very young larvae; (2) a Teleost fish which eats the mollusc, and in which the Plerocercoid larva is found; (3) an Elasmobranch which eats the Teleost and in which the mature Tetrarhynchid is found. Now the difficulty of proving the transmission of the Tetrarhynchid from one to another of these animals has led Southwell to suggest that the Teleost is not a necessary host in the life-history of *T. unionifactor*, but only a "cul-de-sac, or blind termination." The Teleosts and Elasmobranchs alike become infected by eating the molluscs.

Whatever may be the case in the Ceylon species I think that the life-history of *Tetrarhynchus erinaceus* is probably similar to that suggested by Southwell and that some invertebrate is the true larval host. The species of Teleosts mentioned in this paper are not therefore larval hosts in the sense that they are stages in a life-history. They are collateral hosts with the Elasmobranchs; and both series of fishes are infected by eating the true larval hosts—whatever these may be. The mature *Tetrarhynchi* are never found in the Teleosts and larvae entering these fishes finally degenerate[1].

III. *The Plerocercoid larvae.*

The cysts which contain the larvae are either spherical or oval bodies embedded in the outer wall of the alimentary canal and covered by peritoneum; or they are stalked, egg-shaped bodies attached to the intestine or mesentery by means of delicate pedicels. In the Gadoid larval hosts the cysts are usually situated in the outer wall of the stomach, and this is also their site exceptionally in Pleuronectid fishes. The cases of the Halibut and Hake already referred to are the only instances which I have seen of the infection of the muscles with the larvae. In these cases the larvae were enclosed in lenticular cysts which lay compressed between the muscle bundles; or they were cylindrical worm-shaped bodies usually without scolices and filled with calcareous corpuscles and connective tissue, and lying in little tubular cavities hollowed among the muscle bundles. In Gurnards and some other fishes the cysts are rounded or irregular in shape, less usually flask-shaped, and they are nearly always situated on the mesentery and hanging freely into the body cavity. The flask-shaped cysts appear to be the more typical form, and these are always attached to the mesentery by a short stalk which forms the neck of the flask. The size of the cyst is very variable, being anything from 1 mm. to about 1 cm. in length.

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[1] The degeneration of the larvae is accompanied by the progressive accumulation of the calcareous corpuscles. In a Megrim (*Arnoglossus megastoma*) examined since writing the above, living Plerocercoids were present among the muscles of the host. But there were also many groups of prismatic crystals of calcium carbonate lying freely among the muscles, these crystals were in some cases 1 cm. long and about 0·5 mm. in diameter. I think they were the result of the crystallisation of masses of calcareous corpuscles resulting from degeneration of the larvae.

The cysts are often degenerate so that on dissecting them no trace of larval structure can be made out. I have described such structures as occurring in the Gurnard (1905). In these cases the cysts were smaller than usual, round or oval in shape, and pigmented dark-brown towards their central portions. On cutting sections they were seen to be made up of a number of concentric laminae, composed of some substance staining dark blue with Mann's methyl-blue-eosin, and built up of bundles of wavy fibres. In the centre was a nucleus consisting of granules, and on the periphery was a layer of tissue continuous with that of the host, and containing masses of darkly staining granules. The whole structure exhibited a striking resemblance to that of the pearls found in the common edible mussel, the appearance presented by a meridional section reproducing almost exactly the appearance of a decalcified pearl. The origin of these pearl-like cysts in the Gurnard is very probably similar to that of the calcareous pearl in *Mytilus*, that is, they are artifacts produced by specific stimulation of the tissues of the host by some substance excreted by the parasite. In the mussel pearl this substance is excreted by the larva of the Trematode which is responsible for pearl production, and since the parasite inhabits the shell-secreting mantle lobes, the substance which is secreted round the larva is lime laid down in the same way as that forming the nacreous layer of the shell. In the case of the pearl-like bodies in the Gurnard the substance laid down round the larval Tetrarhynchid is a waxy secretion superposed on the fibrous tissues of the larval cyst. (See Ward, 1907.)

The cysts of *Tetrarhynchus erinaceus* and the mode of development of the Plerocercoid larva into the segmented worm have been described by P. J. van Beneden (1850, Pl. XXXIII) but I give here some figures less diagrammatic than those quoted. Text-fig. 1 represents several cysts dissected out from the mesenteries of a *Trigla,* and some other hosts and Text-fig. 2 represents a section through one of these structures taken from a Megrim (*Arnoglossus laterna*) and cut parallel to the plane in which the larva lies. The whole structure represented is the larva. We see that the scolex with its proboscides invaginated lies within a cavity at one pole of an egg-shaped body, and is coiled up in a S-shaped form. The whole scolex is invested in the cuticle characteristic of the Cestodes, and at the base, just posterior to the proboscis bulbs, this cuticle is thickened and becomes continuous with

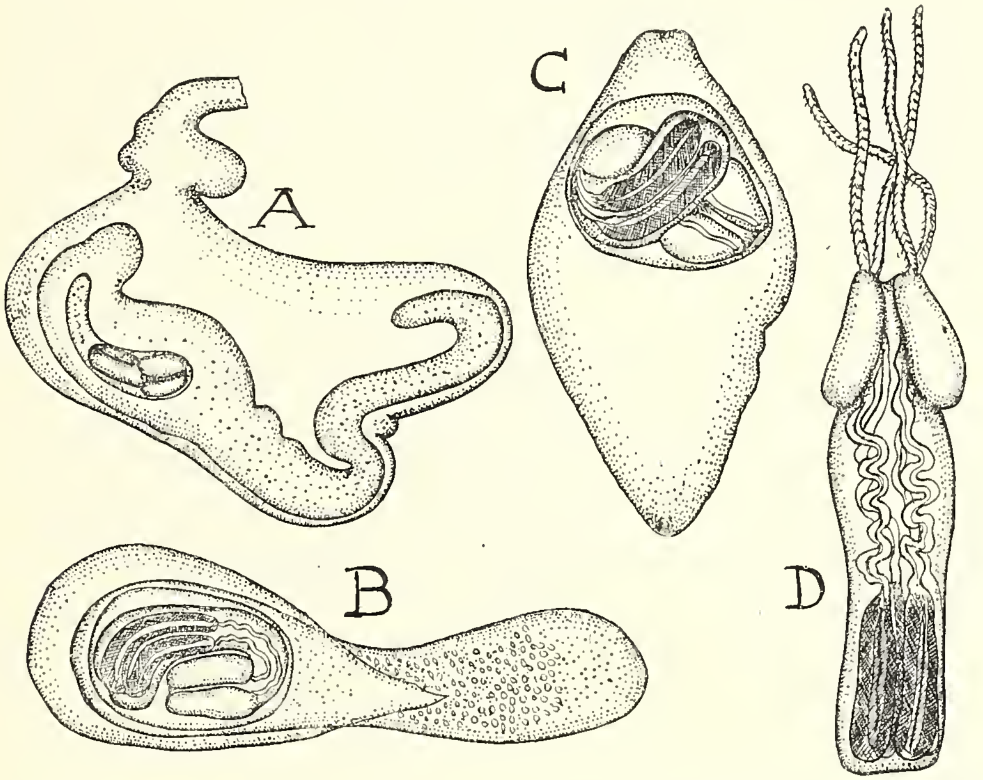

Fig. 1. Cysts containing Plerocercoid larvae of *Tetrarhynchus erinaceus.* A, B and C represent the entire cysts; D is a larva dissected out from the receptaculum. From various hosts.

the internal wall of the cavity in which the scolex lies—the receptaculum scolicis. The internal wall of the latter is not cuticularised except immediately round the base of the scolex. The latter has all the structure characteristic of the mature Cestode and need not be described here. Just anterior to the place where the cuticle becomes continuous with the wall of the receptaculum the excretory canals come near the surface and pass out into the superficial parts of the posterior portion of the larva. This posterior part—the so-called "post-cephalic segment"—corresponds to the bladder of the cysticercoid larva of a *Taenia* but unlike the latter it is not hollow, but is filled with an obscure, faintly-staining tissue which resembles the parenchyma found in the tissues of the Cestodes; and is apparently reticular in nature, with very large interspaces. The excretory canals run backwards, loosely convoluted, in the superficial tissues of the "bladder" and open into a minute vesicle which again opens on to the exterior extremity of the larva. No other structures are present in this posterior part of the larval body.

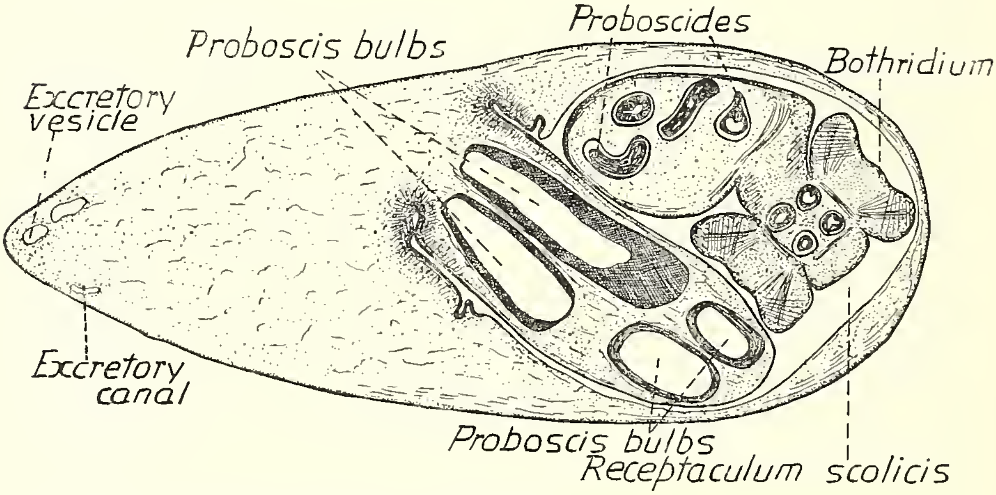

Fig. 2. Longitudinal section of a Plerocercoid larva from the mesentery of *Arnoglossus laterna*. Mag. 50 dia.

The receptaculum scolicis is really an invaginated part of the outer wall of the larva, as one sees in van Beneden's schematic figures. From the bottom of this invagination the scolex forms as a bud, with the bothridia and other structures in their definite relations, and not with their surfaces reversed as in the case of a *Taenia*. There should be an opening in the wall of the receptaculum through which the scolex is pushed when the larval metamorphosis takes place—but not evaginated in the strict sense of the word. I have seen larvae with the scolex pushed out and still adherent to the "post-cephalic segment" but in two series of sections there is no trace of the opening. If we take a globular cyst and dissect it with needles first of all an outer covering comes away—this is partly larval but must also be made up in part of tissues from the host. Within this outer cyst is the larva proper. If one dissects the latter in the same manner an apparent outer capsule can be picked away, and within this is another vesicle containing the scolex. What we first dissect away is the post-cephalic part of the larva and the inner vesicle is the receptaculum.

In some cases the scolex is relatively large, may be coiled up in a double S-shaped form, and may lie apparently quite freely within the receptaculum. I believe that this is the case in the older Plerocercoid larvae: that the opening of the receptaculum may close up completely; that the scolex may become separated from the other part of the larva; and may only be liberated when the double or treble cyst round it is digested within the alimentary canal of the final host. I have never seen any indication of beginning segmentation in the oldest of these Plerocercoid larvae. Obviously very many of the larvae must degenerate since not all their hosts have the evil fortune to be eaten by the final hosts. So one finds cysts which indicate that they are the degenerate remains of larvae only by the presence of the calcareous corpuscles, or by that of some waxy substance.

The Oncosphere larvae, when liberated from the digested proglottis, probably pass through the wall of the stomach or intestine and enter the blood vessels. Usually the latter will be small factors of the hepatic portal system, so that the larvae must find their way through the liver and so into the systemic circulation, and in this way they will be carried to remote parts of the body of the host. One would conclude that a general infection must often take place; nevertheless, it is very rare and the reason may be that the larvae degenerate in the arterial blood stream: it may be that they are obligatory anaerobes. In the great majority of cases the infection is that of the peritoneum. The larvae in such cases have doubtless been carried into the arterioles of the peritoneum or mesenteries, and then they become arrested by the decreasing calibre of the arterial twigs in which they are circulating. They then begin to grow in size occluding the blood vessel, and producing a nodular swelling on the course of the latter. If the vessel lies near to the surface in the peritoneum covering the stomach or intestine, or if it is situated in a mesentery, the nodule will come to project into the body cavity and a short stalk will be formed, so that the typical flask-like larval cyst is formed.

Summarising the life-history we find that (1) *Tetrarhynchus erinaceus* is sexually mature in the large intestine of various species of *Raia,* but not in other genera of fishes so far as our knowledge goes. These fishes act as the "final host" and the ripe proglottides are then expelled *per anum* with the faeces. (2) The proglottides may lead an independent existence for a short time at the sea bottom and are then probably eaten by some small fish or invertebrate—the true larval host. (3) This animal is then probably eaten by the collateral host and the Oncosphere larvae develop within the body of the latter, usually inhabiting the peritoneum over the alimentary canal, or in the mesenteries; and there they attain the Plerocercoid stage and degenerate. Or the larval host may be eaten by the final host and the larva then begins to segment and attains sexual maturity.

IV. *The scolex.*

Tetrarhynchus erinaceus as found in Irish Sea fishes is rarely more than about 60 mm. in total length, but the ultimate proglottides are continually dehiscing from the strobila and one or more may usually be found in the large intestine of the host. The mature Cestode will live at least for several hours in a dish of clean sea water. It is very mobile, drawing itself along by means of rhythmic movements of the bothridia. The latter elongate, their anterior extremities being pushed forward and fixed to the object over which the worm is moving; the posterior extremities are then drawn forward and the scolex fixes itself in a new position. The proboscides act apparently only as organs of attachment and do not take an important part in the locomotion of the worm. The Cestode is rapidly killed by immersion in fresh water, and it soon breaks up when preserved in weak formalin. When preserved in any of the mercuric chloride fixatives the strobila usually remains intact.

The worm represented in Pl. XIX, fig. 1 was about 52 mm. in total length and possessed 47 distinct proglottides. The most anterior segments are very indistinct: they are about 0·2 mm. in length and about 0·6 mm. in breadth. The terminal proglottis, which was still incompletely ripe in the specimen figured, was about 3 mm. in length, and about 1·2 mm. in breadth. The anterior segments are elliptical in transverse section, being slightly flattened dorso-ventrally[1] but the more terminal segments are very nearly circular in transverse section. The genital apertures may usually be seen on the latter ten or so segments. They are always lateral in position and may be present on either margin of the proglottis but without any regularity as regards the side on which they occur.

Bothridia. The scolex possesses two suckers or bothridia which are set at an angle to each other so that their anterior extremities approximate (Pl. XIX, figs. 2 and 3). They are sessile and their thickened rims are usually all that project beyond the surface of the axial part of the scolex. Seen *en face* they are roughly oval in outline but present a well-marked notch at their posterior margins and a slighter notch at their anterior margins. The suctorial surface is that of a cup marked longitudinally by a shallow fossa. The depth of the suctorial cup depends entirely upon the degree of contraction of the scolex: in Pl. XIX, fig. 2 the scolex is represented in the extreme degree of contraction, while Text-fig. 3 shows the scolex in a state of almost complete relaxation. The marginal parts of the bothridia consist of hard parenchymal tissue but the greater portion of the organ is muscular.

The diameter of the scolex decreases passing back from the bothridia and then increases again, so that a swelling is formed and this marks the positions of the proboscis bulbs. All this part of the Cestode may be regarded as the scolex, or as the scolex and "head-stalk." The neck is the region immediately posterior to the proboscis bulbs and is characterised by lack of segmentation and by relatively undifferentiated tissues. Within it are the excretory canals, the longitudinal muscles and the nerve cords, but there are no traces of genital organs. Of course there is no sharp distinction between the scolex, neck and the anterior segmented part of the strobila. Behind the neck segmentation begins, the junctions of the young proglottides being marked by narrow transparent lines. Then one begins to see the rudiments of the genital organs as cords or blocks of cells.

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[1] The terms "dorsal" and "ventral" have not the same precise significance in the case of a tapeworm as in the case of a higher animal. The ventral surface is usually regarded as that on which the genital aperture is situated, but this is lateral in *T. erinaceus* and one can only fix the ventral surface by a comparison of the relations of the genital organs. The bothridia are dorsal and ventral, but I have tried to avoid making any distinction between upper and lower surfaces and use the term dorso-ventral to designate the diameter at right angles to the diameter—from side to side—which is in the plane in which the lateral excretory canals are situated.

The proboscides. These are the most characteristic organs of the Cestode and enable one to identify it at a glance. They are differentiated very early in the development of the scolex, even before the bothridia have been formed, and the identification of the larva is always an easy matter when the proboscides can be seen. Usually they are invaginated in the larva, if so they can sometimes be extruded by gentle pressure on the cover glass while the worm is alive: but even if they are not extruded the large hooks can usually be seen. They are nearly always evaginated in the living worm and are extruded when the latter is killed by immersion in fresh water. There are four of these structures; and this number, with the presence of the notches and fossae on the bothridia, indicate that the primitive number of bothridia was also four. In a large *Tetrarhynchus erinaceus* the proboscides may be from 2 to 4 mm. in length and from 0·4 to 0·5 mm. in total diameter. Each proboscis consists of a sheath continuous from the tip of the organ to its insertion in the proboscis bulb. The sheath consists of two parts—proximal and distal. The posterior or proximal part is an integral part of the tissues of the scolex, is non-evaginable and is unarmed. The distal part is evaginable and is beset with hooks and spines on its internal surface. When it is invaginated it lies within the proximal part, but when it is evaginated the surfaces are reversed so that the hooks lie on its outer surface.

The sheath of the proboscis is, in life, a clear, hyaline, tubular structure. The evaginable part consists of one uniform, structureless layer in which are inserted the hooks and spines. The part which lies within the scolex and is non-evaginable consists of two concentric laminae differing slightly in staining reaction. These layers are indistinctly separated from each other after fixation and the external one may exhibit an apparent vacuolisation. There are no muscle fibres on the sheath but here and there one may see scattered nuclei. The proboscides emerge from the scolex at the anterior and lateral margins of the bothridia, and at these points the tissues of the sheaths become continuous with the cuticle investing the body of the Cestode.

The armature of the proboscis differs strikingly from that of other Tetrarhynchids with which I am acquainted and I propose to describe it in some detail since neither van Beneden (1858, p. 128, Pl. XVII) nor Vaullegeard (1899, Pl. XV) figures it very accurately. Pl. XX, figs. 6 and 7 represent reverse and obverse views of the same proboscis (taken from a mature worm) and fig. 8 represents one view of a proboscis taken from a larva inhabiting the muscles of the Halibut. There may be some slight variation in the arrangement of the hooks when the larva and adult worm are compared, but as a rule there is a great constancy in mature individuals. In comparing these drawings with actual specimens one must remember that quite a different appearance will be presented according to the way in which the proboscis lies since the hooks and spines in each oblique series differ from each other. One cannot slit up the proboscis and spread it out on the flat; and since it is necessary to focus down to the level of each hook it follows that those at the margins of the proboscis must be foreshortened. I have not been able to disintegrate the proboscis by maceration, or by treatment by alkalies: what happens in the latter method of treatment is the solution of the spines, and the shrivelling up of the sheath.

The hooks and spines are arranged either as a continuous spiral running round the proboscis, or as a series of obliquely running rows—I have been unable to determine which arrangement is the actual one. They must be repeated from base to tip of the proboscis with great exactness since one always finds the same kind of hooks at the margins throughout a considerable part of a proboscis. There are five different kinds of hooks and spines which I letter as follows—the lettering being similar to that of the figures on Pl. XX.

A. Large, broad, sharply pointed hooks with slipper-shaped bases. These hooks are remarkably constant in size and shape and measure about 0·06 mm. from the perpendicular through the tip to the perpendicular through the other extremity of the base. There are two of these hooks in each series, and they lie adjacent to each other.

B. Long, relatively narrow, curved spines with small expanded bases. They are nearly straight for the greater part of their lengths but are sharply recurved at the tips. Fig. B in the Plate represents the extreme degree of curvature, usually the hook is less sharply curved. The length is about 0·04 to 0·05 mm. measured as in the figure. There are two of these hooks in each series, one adjacent to each of the large hooks A.

C. Relatively long spines with slender sharp tips, and blunted projections near the bases. The latter are very small. The apical part is bent round nearly at a right angle to the basal part and is sometimes recurved in the opposite direction. The projection near the base may function like the barb in a fish-hook. The length of these hooks is about 0·04 mm. and there are two in each series, one being adjacent to each of the spines B.

D. Long, very slender spines about 0·06 mm. in length. Their bases are only slightly expanded and the spines themselves are very nearly straight. There are two of these spines in each series, one adjacent to each of the hooks C.

E. Small, slightly curved spines about 0·01 to 0·02 mm. in length. These spines are rather variable both in size and shape. Sometimes they are relatively long and resemble the spines D. Such are those found in the region beneath the large spines B to D where they are arranged in two rows of about six in each. Other spines are short and have broad bases being rather like the large hooks A except that they are very much smaller and not so strongly bent. In profile they are as represented in E, fig. 9. These spines are arranged in a continuous longitudinal series of several rows between the two spines D.

In all these spines the curvature is outward and backward. All are hollow with openings at their bases. There does not appear to be any mechanism for their regeneration. Their general arrangement is represented in the following table where size of the letters represents roughly the relative sizes of the spines.

EEE						EE
EEE	D	C	BAAB	C	D	EE
EE						EEE
EEE		EEEE		EEEE		EE

The Musculature of the Scolex. The muscles of the scolex may be arranged in three main categories: (1) the complex musculature of the bothridia; (2) the extrinsic muscles of the proboscides; and (3) the intrinsic muscles of the proboscis. Both bothridia and proboscides are organs of locomotion rather than of attachment. The Cestode is always situated in the large intestine of the host behind the folds of the spiral valve, and thus in such a situation that it is not likely to become detached by the movement of food matter passing through the intestine. Therefore it is generally very lightly attached and can easily be removed without injury to the tissues of the scolex. The bothridia are used to enable the worm to execute creeping movements on the wall of the intestine in the manner already described. The proboscides appear to be used for the same purpose: first of all the organ is extruded and the hooks adhere to the intestine. It is then invaginated and the scolex is thus drawn forward. Both the bothridia and proboscides are therefore very mobile and have a rich muscular supply.

The Bothridia Muscles. Pl. XIX, fig. 4 represents a transverse section through the anterior part of the scolex. The bothridia are seen to be two shallow suckers, the marginal parts of which are composed of dense parenchyma and muscle fibres. The central parts are mainly parenchymatous but contain also some muscle fibres with other tissues. Towards the lateral margins, and placed symmetrically with regard to each other, are the proboscis sheaths. The proboscides themselves are evaginated and the structures within the sheaths are the retractor muscles.

It is difficult to represent the exact dispositions of all the systems of muscle fibres in such a figure as this so I have prepared a diagram which shows the various components of the musculature of the bothridia as they would be seen if projected in a single transverse plane. They form a group of mutually antagonistic muscles the action of which permits of a great variety of movements on the part of the bothridia. They may be arranged as follows, the lettering of the various paragraphs corresponding to that of the diagram.

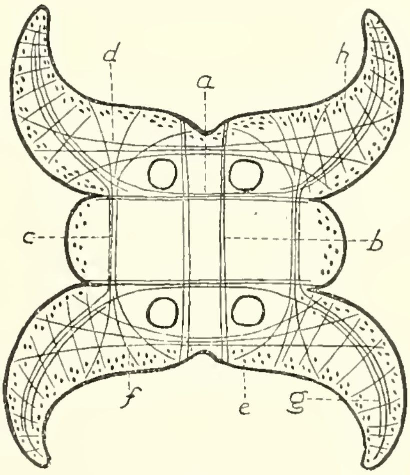

Fig. 3. Diagrammatic transverse section of the scolex representing the arrangement of the muscle fibres. Only a few fibres of each series are figured.

a. Transverse fibres running straight across from side to side of the scolex in two parallel bundles, one of which is internal to each dorsal and ventral pair of proboscides. They are inserted into the dense parenchymatous tissue at the places of origin of the bothridia.

b. Transverse dorso-ventral fibres which run at right angles to those lettered *a*, and which are arranged in two bundles or sheets, one of which is internal to each lateral pair of proboscides. They are inserted into the integument of the suctorial faces of the bothridia on each side of the shallow fossae which run longitudinally along the faces of the latter organs.

c. Transverse fibres running chiefly dorso-ventrally in the parts of the scolex external to the proboscides. They form two compact sheets of fibres in the axial parts of the scolex but fan out in the bothridia so that their fibres are inserted in the tissues beneath the integument of the suctorial faces between the longitudinal fossae and the lateral margins.

d. Tangential fibres which are gathered up into two compact sheets in the central parts of the scolex, and which run roughly parallel to the central parts of the suctorial faces of the bothridia. Laterally these fibres radiate out and are inserted into the integument of the external convex faces of the bothridia.

f. Fibres inserted in the marginal parts of the suctorial faces of the bothridia. Some of these run dorso-ventrally from one bothridium to the other, and are thus identical with series *c* but others originate in the dense tissue where the bothridium arises from the axial parts of the scolex.

g. Radial fibres running in sheets parallel to the marginal parts of the bothridia and inserted into the tissues forming the rims of the latter. Some of these fibres originate in the dense tissue at the bases of the bothridia, but others are identical with the fibres of series *a* and *c*.

h. Numerous fibres which join the suctorial (concave) and external (convex) faces of the bothridia. They may function in flattening the latter organs.

e. In addition to all these fibres there are others which run longitudinally beneath the integument both in the bothridia and in the axial part of the scolex. Those which are present in the bothridia are evidently inserted into the dense tissue at the anterior and posterior ends of these organs, but those present in the axial part of the scolex are an integral part of the general longitudinal series of integumentary muscles of the whole strobila.

All these muscles fibres are smooth and unstriated and are not provided with nuclei nor have they any myoblast substance. They are about 2 μ in diameter, stain deeply with methyl-blue-eosin and can easily be distinguished from the surrounding tissues.

The Muscles of the Proboscis. The extrusion of the proboscis is not directly a muscular act. The lumina of the sheaths and bulbs are filled with fluid and the extrusion of the proboscides is the result of an increase in the pressure of this fluid. The lumen of the sheath and that of the bulb are continuous so that when the latter is contracted the fluid in the sheaths presses upon the part of the wall just where it is invaginated inwards. The mechanism involved is obviously similar to that which leads to the evagination of the proboscis in Nemertine and Acanthocephalan worms.

There are four series of muscles in relation to the proboscides:—

(1) Extrinsic tangential fibres. These are represented in Pl. XIX, fig. 4. They are found in the region of the non-evaginable proboscis sheaths. They are fibres which originate in the walls of the bothridia and the posterior part of the scolex and which are inserted tangentially into the walls of the sheaths throughout the greater part of the lengths of the latter. Their function is obviously that of holding in position the non-evaginable parts of the proboscis sheaths.

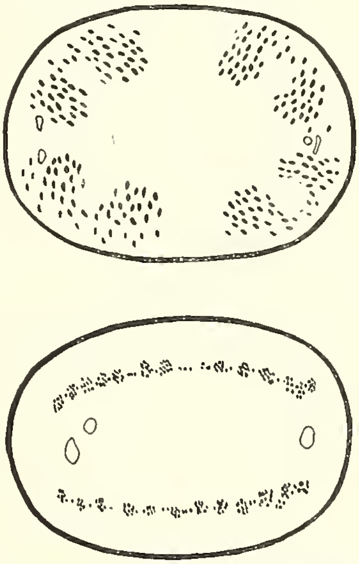

Fig. 4. Two diagrammatic transverse sections through the neck of the worm. The upper section passes immediately behind the proboscis bulbs; the lower is just before the region of segmentation.

(2) Extrinsic longitudinal fibres. These are represented in Pl. XIX, figs. 3 and 5 and in Text-fig. 4. They are relatively coarse fibres which are inserted in the outer parts of the walls of the bulbs along the greater parts of the length of the latter. Towards the posterior part of the bulb these fibres are gathered up to form four roughly crescentic bundles the convex surfaces of the crescents being external (Text-fig. 4).

A little way further back in the neck of the Cestode these bundles flatten out and the two dorsal ones fuse together to form a sheet of fibres, while a similar fusion takes place with respect to the ventral fibres. (Fig. 4, the lower figure.) They now form the anterior section of the general longitudinal musculature of the strobila.

(3) Intrinsic proboscidial muscles. The proboscis bulbs are the posterior terminal parts of the proboscidial apparatus, and are situated near the posterior part of the scolex at the place where the latter exhibits a distinct swelling. They are represented in Pl. XIX, fig. 3 as they are seen in cleared preparations of the scolex, and they are also represented in section in Pl. XIX, fig. 4. Each of them is a cylinder which is about 2 mm. in length and about 0·7 mm. in diameter. The bulb is nearly circular in transverse section and consists of an outer wall of cuticular nature which is continuous with the wall of the sheath and with that of the proboscis; of an inner wall, also continuous with the sheath; and of an intermediate layer of muscle fibres. This muscular part takes up by far the greater part of the thickness of the wall of the bulb: it is not of uniform thickness and fails entirely at the part of the wall nearest to the integument of the scolex and just here the cuticular outer part of the wall of the bulb is greatly thickened and forms the place of origin and insertion of the muscle fibres composing the intermediate layer. The diameter of the lumen of the bulb is only about one-third of the total thickness of the latter, and the lumen itself is not quite circular in transverse section.

The muscle fibres included in this intermediate part of the bulb wall are arranged in two series which run obliquely round, being inserted at either end into the thickened cuticular external wall. They continually decussate with each other crossing at an angle of about 45°. They are not arranged in bundles or laminae but lie quite loosely from each other except at certain places where the fibres appear to be twisted round each other. Obviously the effect of the contraction of these muscles will be to reduce the diameter of the bulbs and so to exert pressure on the fluid contained in the lumina of the sheaths.

(4) The Retractor muscle of the proboscis. This structure (see Pl. XIX, fig. 5) is a compact bundle of smooth fibres each of which is about 2 μ in diameter, the whole bundle measuring about 0·03 mm. in total thickness. It originates at the extreme posterior end of the proboscis bulb and is inserted into the internal wall of the proboscis at the extreme tip of the latter. The muscles run axially through the sheaths and bulbs and are quite free from the latter except at their ends. They must be capable of contraction to about one-third of their entire lengths. They function of course in the invagination of the proboscides.

The supposed striation of the muscles of the proboscis bulbs. A distinct striation of these fibres has been described by several authors. Pintner (1880, p. 51) states that they are generally striated and comments on this condition as one of the very few instances of striped muscle in the phylum of the Platyhelminthes. He describes the proboscis bulb in *Tetrarhynchus longicollis* van Beneden as being built up of six distinct laminae in all of which the fibres are transversely striated; and he figures an isolated "Muskelband" in which there is a very evident appearance of banding (Taf. IV, Fig. 6). The diameter of this structure is not given so that it is difficult to be sure whether a single fibre or a small group of such is intended to be represented. In a further paper Pintner (1893) describes a similar condition in *Tetrarhynchus smaridum* Pintner, and though he admits that the striation is "ausserordentlich Zarte," and only to be seen with difficulty, his references and figures (p. 626 and Taf. IV, Fig. 60) appear to leave no doubt as to his interpretation of the structure seen in the fibres. Vaullegeard (1889, p. 316) describes striated fibres in the bulbs of *Tetrarhynchus rufficollis* Eisenhardt; and Lönnberg (1889) describes a similar condition in the larval form *Coenomorphus linguatula* (van Beneden) and in *Tetrarhynchus tetrabothrius* van Beneden (Lönnberg, 1891, p. 60, Taf. I, Fig. 17). There is therefore an apparent body of evidence in favour of the occurrence of cross-striated muscles in Tetrarhynchids. Now it appears unlikely, *a priori*, that transverse striation, which is usually associated with rapidly contracting muscle fibres, should occur in the slowly contracting muscles of the proboscis bulbs and be absent in the much more mobile bothridial muscles, and it is worth while examining further into the nature of these structures. I am quite unable to find any evidence that such indubitably striated fibres do occur either in *Tetrarhynchus erinaceus* or in *T. tetrabothrius* and I can only explain the strikingly distinct figures given by the authors quoted by supposing that they have been observing artifacts. Pintner's work on the Tetrarhynchids bears every indication of extreme accuracy of observation and I have been able to confirm almost every detail of structure described by him, with the exception of these so-called striated muscle fibres; and must conclude that the appearance is a spurious one.

Such a spurious striation certainly occurs in the species now under description and is represented in Pl. XXIII, figs. 22 and 23. It will be seen from Pl. XIX, fig. 5 that the decussation of the muscle fibres is exhibited very clearly in certain parts of the transverse section of the bulb. In such a section the fibres are either cut obliquely so that short lengths of each are to be seen in the same field, or they may be cut so that comparatively long lengths are shown. As a rule the fibres lie quite loose from one another, especially when the proboscides are retracted, and the muscles of the bulbs are relaxed; but when the latter are contracted so as to produce the evagination of the proboscis quite a different appearance may be observed, and then an apparent cross-striation, but altogether too bold and striking an appearance to be a genuine one, may be seen. I have represented this appearance as seen under an oil immersion lens in Pl. XXIII, figs. 22 and 23. The former figure suggests that there are alternate light and dark fibres running parallel to each other and that while the darker fibres are not marked in any way the lighter ones are most distinctly crossed by transverse lines, each line indicating a constriction of the fibre. This appearance is as a rule most marked at the part of the wall of the bulb nearest to its internal surface (with respect to the axis of the scolex), and here the fibres may appear to be cut up into blocks. The latter figure represents a part of the muscular wall of the bulb near to the place of insertion of the fibres in the cuticularised external surface and here the fibres are obliquely striated so that they resemble closely twisted ropes of several strands. The diameter of these "fibres" is about 2–3 μ.

I think it is quite evident that this so-called striation is really due to an interlacing of the fibres so that strands of two or more are formed. If this interlacing were very close the fibres might appear to cross one another at a right angle and so we should have an appearance of striation, but obviously a spurious striation. The proof of a real banding would be afforded by the staining of isolated fibres, and Pintner indeed figures such a dissociated fibre, but it appears to me to be likely that this structure was really compound. I have tried dissecting out the whole bulb and teasing it so as to break the muscular wall and leave the ends of the fibres projecting. In such a preparation there is no trace of a real striation.

The Excretory Canals. There are two longitudinal excretory canals in the more anterior proglottides on either side, but these vessels branch in the scolex and form a very complex system. The two main canals continue to run as far forward as the posterior extremities of the proboscis bulbs, but about this region they begin to branch so that four or five canals of approximately equal diameter may be seen lateral to the proboscis sheaths. These main canals cannot be seen in the axial parts of the scolex, nor dorsal or ventral to the proboscis sheaths; behind the bothridial region. Their diameter varies very greatly so that sometimes they are large and quite circular in transverse section, and possess very thin walls with very few nuclei, but at other times they are greatly contracted and appear as mere chinks in the tissues of the scolex, their walls then being thick and rugose in transverse section. Occasionally the canals may be so much contracted as to be almost invisible so that it is very difficult to trace them from section to section of a series. The varying diameter appears to be due mainly to the manner of fixation of the worm. The distribution of the main excretory canals in the bothridia is very complex since they form a close plexus here. The main canals branch repeatedly and the finer vessels are connected by numerous anastomoses. These smaller branches are most abundant in the neighbourhood of the central nervous system and in the marginal parts of the bothridia particularly underneath the suctorial faces of the latter.

The Central Nervous System. There is greater difficulty in the investigation of the nervous system than in that of any other system of organs in the whole Cestode. This difficulty is, of course, one which is experienced by all students of the nervous system of invertebrata, and depends largely on the failure to obtain a thoroughly satisfactory staining reaction in the case of the fibres. But added to this we have in the case of the Cestodes an arrangement of nerve tracts and centres which is unfamiliar since it differs notably from the conditions in most other invertebrates; and which appears to be remarkably complex when one considers that there is not the same necessity for coordinated movement on the part of the animal, while the environment is a very uniform one. Added to these difficulties there is that of the lack of definiteness in the centres and nervous tracts. In most invertebrates one finds rigidly circumscribed, or encapsulated centres or ganglia, and compact nerve cords; but in *Tetrarhynchus* all the organs are embedded in the ubiquitous parenchyma, and sometimes the limit between this tissue and the organs which it surrounds is not a very well-defined one.

General stains do not differentiate the nervous structures of this tapeworm in a very satisfactory manner; and in the only preparation which I have been able to make, involving the use of vom Rath's fixative followed by staining in iron haematoxylin, the reaction is not much more useful. Most of the figures which I give here are based on one complete series of sections of a scolex taken from a worm which was killed in fresh water and fixed in dilute formalin. These sections were stained with methyl-blue-eosin, but probably because of the imperfect fixation the usual purple of the sections faded entirely and left only a pure blue. It was evident that only the modified parenchyma in the nervous system had stained, with of course the muscle fibres in surrounding parts.

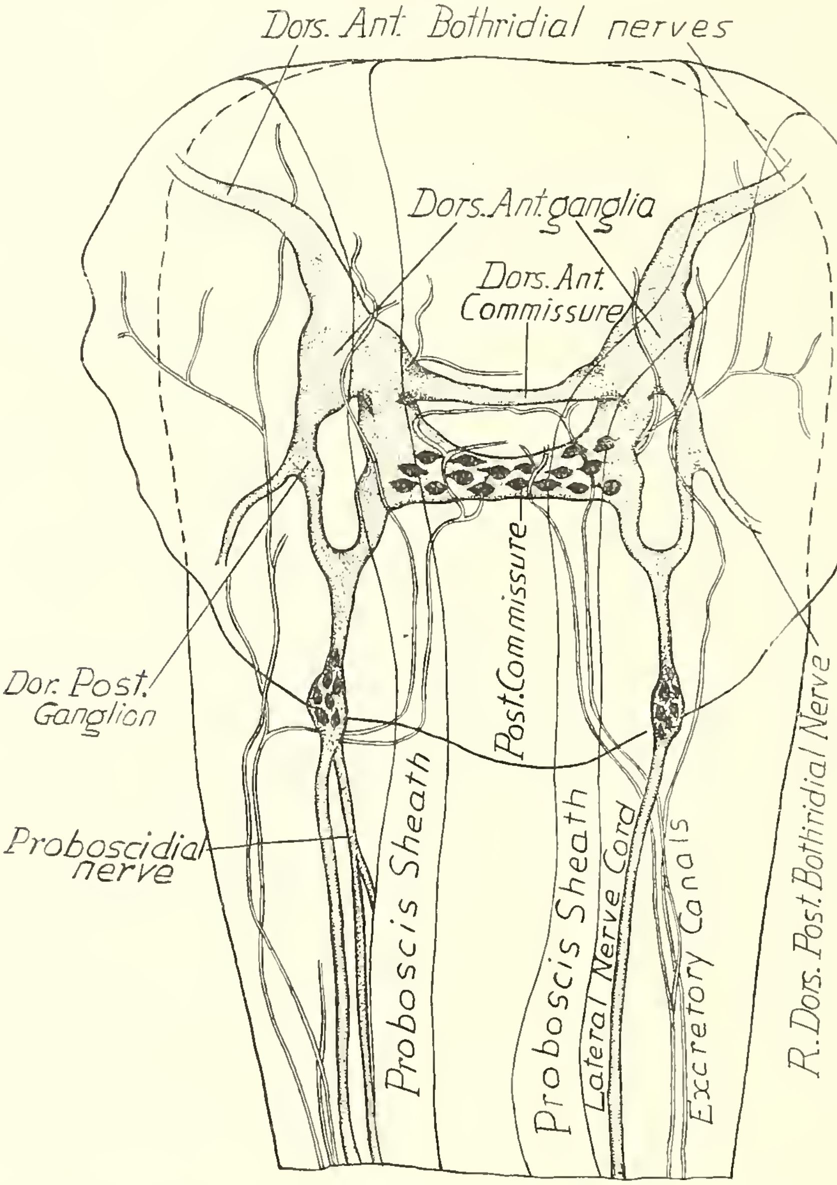

Fig. 5. Schematic representation of one-half of the central nervous system seen as projected in a horizontal plane. The relative proportions of the various parts are fairly accurate, the figure having been reconstructed from serial sections. The darkly shaded parts are the approximate loci of the ganglionic cells. The main trunks of the excretory canal system are also shown. Mag. 90 dia.

In several other series of sections fixed with Zenker's fluid and stained with methyl-blue-eosin a finer differentiation was obtained but the staining was then much more general and it became exceedingly difficult to trace the boundaries of the nerve tracts and centres, though details of structure became apparent. I have therefore reconstructed the nervous system of the scolex from the former series, which although defective as regards the differentiation of the essential nervous elements still enables one to trace the *locus* of the organs. Fig. 5 is a projection of the dorsal half of the central nervous system in one horizontal plane,

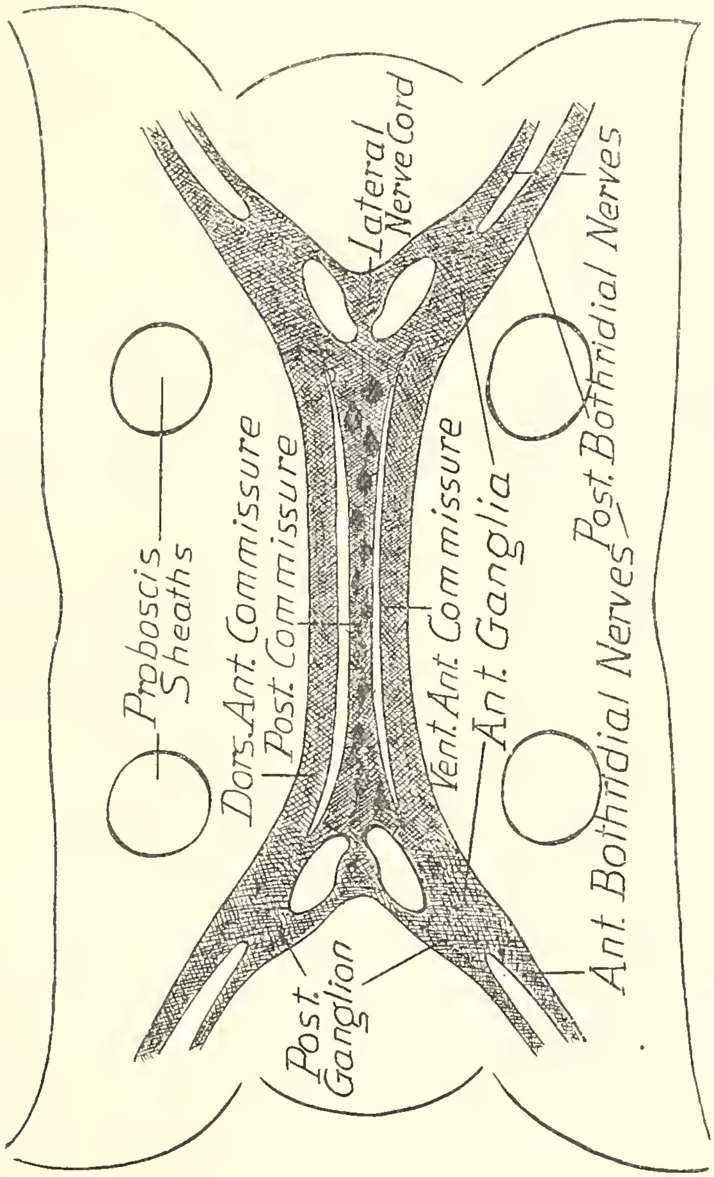

Fig. 6. The central nervous system. Schematic, but the proportions are fairly accurate. The darkly shaded part contains ganglion cells.

and has been reconstructed from serial sections; and Fig. 6 is a similar reconstruction projected in a single transverse plane. Fig. 7 is a pure diagram but may be useful in enabling the reader to follow the description. The exact situation of the central nervous system may be fixed by a reference to the diagrammatic transverse section (Text-fig. 3, p. 379). It is contained in the space bounded by the dorso-ventral muscles *c*, the horizontal muscles *a*, and lying between the dorsal and ventral pairs of proboscis sheaths. It is all contained within relatively few sections of the series.

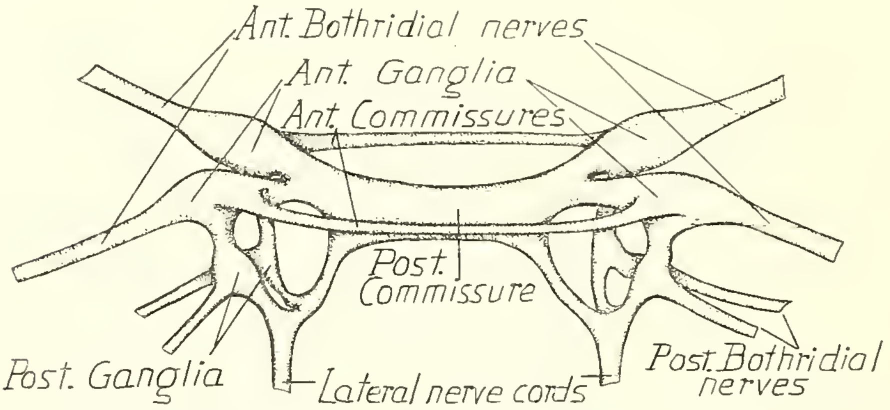

Fig. 7. The central nervous system. The figure is entirely schematic, the commissures being rather widely separated so as to make their relations as clear as possible.

The whole is built up round four paired masses which in order to avoid circumlocution I will call ganglia. These structures are arranged as right and left dorsal, and right and left ventral. From each of them a stout nerve, hardly less in diameter than the ganglion itself, proceeds and runs transversely and anteriorly into the antero-lateral parts of the bothridia. These are the paired anterior bothridial nerves—four in number. The ganglia are connected together by commissural tracts. The corresponding right and left centres are joined together by paired transverse commissures. These two commissures run transversely across the scolex from side to side and near to the middle line—much nearer than is suggested in the diagram Fig. 7. Posterior to them the anterior ganglia of the same side approach each other in the middle line, and right dorsal and right ventral masses fuse together, also left dorsal and ventral centres. From the points of fusion another commissure takes origin: this is the posterior median commissure: it crosses the scolex from side to side just posterior to the anterior tracts. Thus we have the paired ganglionic masses connected together by means of three commissures—two anterior and paired, and one posterior and unpaired.

Each of the four anterior ganglia is continued backwards in the form of a very short longitudinal connective. Almost immediately after springing from the anterior ganglia each of these connectives swells out so as to form four posterior ganglia and from each of these masses a nerve takes origin: the four nerves so formed are the posterior bothridial nerves: they run backwards and outwards to the postero-lateral parts of the bothridia.

The longitudinal connectives are then continued backwards and the two on each side fuse together and are joined by another connective which proceeds from the lateral margins of the posterior commissure. The three connectives of each side now fused together form the lateral nerve cord and this passes posteriorly along the scolex. Presumably it runs backwards and is continuous throughout the strobila from segment to segment, though I have not been able to identify it with certainty in the sections of the proglottides. Just opposite to the posterior margins of the bothridia, but within the axial part of the scolex, this lateral nerve cord swells out again to form what appears to be a short columnar ganglion, and a stout nerve takes origin in this structure. The two nerves so formed—the proboscidial nerve trunks—run backwards and inwards toward the axis of the scolex and very soon bifurcate. The two branches of each side run posteriorly, still enclosed in the same sheath of parenchyma between the proboscis sheaths, and at the beginning of the proboscis bulbs they separate from each other. Along that part of the outer wall of the bulb which is nearest to the axis of the scolex there is a strip of tissue of very obscure nature, consisting of parenchymatous and large rounded cells. The proboscidial nerves apply themselves to these strips of tissue and presumably the fibres penetrate into the muscular wall of the bulbs. It is quite unknown in what manner the finer fibres of the nerves end in the muscle fibres of the bulb, and I have, as yet, no observations to make with regard to this point.

Now so far there is little doubt as to the relations of the various parts of the central nervous system which we have been considering. But whenever we begin to examine more closely into the minute structure of the masses called "ganglia" and "commissures" we encounter difficulties. The ganglia ought obviously to contain nerve cells, unipolar or multipolar, and the commissural and connective tracts should consist of nerve fibres, or of the ramifications of the processes of nerve cells. But of nerve cells there is no trace in the so-called ganglia, anterior and posterior. In the series of sections from which Text-figs. 5 and 6 have been reconstructed there are indeed no traces of truly nervous tissues of any kind—neither cells nor fibres. All that is present is a modified parenchyma consisting of a relatively coarse and deeply staining reticulum, the fibres of which are arranged to form a network enclosing polyhedral or rounded spaces, and including very few nuclei within itself. This tissue is very similar in nature to the modified parenchyma which is found beneath the basement membrane of the integument; it is not definitely bounded but is continuous with the general parenchymatous tissue which surrounds the organs of the scolex and proglottides; and it is a supporting structure, or neuroglia, for the essential elements of the nervous system. When the scolex is stained so that the blue colouration obtained from methyl-blue-eosin becomes predominant, and the red and purple reaction is faint, or absent, this neuroglial tissue is all that one sees.

In sections made from a scolex which has been fixed with Zenker's fluid, and stained with methyl-blue-eosin so that the red and purple hues predominate, the appearance of the tissue is quite different. The nervous system is now much more definitely circumscribed and one can make out the existence of a sheath such as is described by Pintner (1880)—a very delicate capsule on which are situated some scattered nuclei. The parenchyma cannot now be distinguished as a separate tissue element. Within this sheath there is—in the structures described as anterior and posterior ganglia, anterior commissures, bothridial and proboscidial nerves, and connectives—only a kind of "neuropil" a fine granular substance staining a faint red with methyl-blue-eosin, and evidently consisting of the sections of very fine fibres cut in all planes. There are no nerve cells in any of the parts of the central nervous system mentioned above; and it is only in the posterior unpaired commissure, and in the short columnar ganglia situated on the courses of the lateral nerve cords, that true ganglion cells are to be found. These ganglion cells are both unipolar and multipolar; they are rather large (about 40 μ in longest diameter) and have all the structure usually associated with such cells in other invertebrates. They are very easily seen in properly stained sections and I have no doubt as to their absence in all the parts of the nervous system except in the posterior commissure and in the secondary ganglia on the lateral nerve cords—the only parts of the whole series which can properly be regarded as nerve centres. Now the use of the terms "ganglion" and "commissure" in relation to the parts of the nervous system of *Tetrarhynchus* appears to be of doubtful propriety, but I am unable to describe them otherwise in few words so as to make their topography clear. Until the whole system of organs shall have been described in the light of information gained from a variety of staining and fixing methods the above terms may suffice.

The most accurate account of the central nervous system of a Tetrarhynchid in the literature is that of Pintner (1881) with reference to *T. longicollis* van Beneden. In a later paper Pintner also describes the nervous system of *T. smaridum* Pintner. This later paper is evidently only a preliminary description but the relationships of the nervous system are essentially those which I have just described with reference to *T. erinaceus*. Pintner's Pl. III, figs. 33 to 47 represent a series of sections through a scolex and one can easily distinguish the four ganglia, the two paired anterior, and the posterior unpaired commissures just as they are represented in the present paper. There is little doubt that the conditions in *T. smaridum* are identical with, or are very similar to, those of *T. erinaceus*. In the earlier paper quoted Pintner gives a much more detailed description of the histology of the nervous system of *T. longicollis* and there is evidence everywhere in this fine memoir of accurate and close observation. The anterior ganglia are represented as fibrous in structure and as entirely devoid of nerve cells; and this is also the structure of the two anterior commissures; but instead of an unpaired posterior commissure Pintner describes a ganglionic mass occupying the same position. The whole brain is described as being surrounded by a sheath, finely fibrous in structure and containing nuclei. It is described in general terms as consisting of a ganglionic centre surrounded by a ring-shaped commissure.

In *Tetrarhynchus erinaceus* this fine structureless membrane, or brain sheath, can just be seen in relation to the anterior ganglia; it does not appear to be present, or it is very obscure in relation to the commissures, while it appears to be certainly absent from the periphery of the posterior commissure. In a few sections made from an imperfect scolex fixed in vom Rath's fluid and stained with iron haematoxylin this posterior commissure is present and I am sure that it contains fibres running longitudinally through it as well as nerve cells, which appear to be multipolar. But the posterior commissure is not nearly so compact as either of the others, and the ganglion cells are surprisingly few in number: in a complete series of sections they could easily be counted. Thus the nervous system differs from that of most invertebrates (1) in that the periphery is fibrous while the central region is ganglionic; (2) in the massive development of the commissural tracts in relation to the truly central elements; and (3) in the curious lack of definite boundaries to all these parts, with the possible exception of the anterior ganglia. It is indeed difficult to be sure of the distinct existence of a definite brain sheath when one fails to delimit it in sections stained so as to bring the parenchymatous matrix into prominence.

I think that the ganglionic region on the course of the lateral nerve cords has possibly been seen by Laczkò (1880). In a paper which is apparently only a preliminary communication this author describes "machtige" ganglionic regions in the nervous system of Tetrarhynchid larvae. Some of these appear to be columnar and to be situated in the neighbourhood of the lateral nerve cords. In the absence of figures it is indeed impossible to be certain as to the precise situation of these structures but it is possible that they are those which I have mentioned as giving origin to the proboscidial nerves.

V. *The integument.*

The membrane covering the outer surface of the body of a Cestode differs notably from that present in most other invertebrates. In such a form as that now being described the tissue elements are: (1) the cuticula; (2) the underlying parenchyma; (3) the subcuticular cells; and (4) the integumentary muscles. A "basement membrane" lying just beneath the cuticle is also frequently described, and the integument also contains gland cells, calcareous corpuscles, myoblasts, nervous elements, and the terminal flame-cells of the excretory organs. I am unable in the meantime to give a detailed description of the latter structures.

The cuticula. This layer covers every part of the surface of the body and is directly continuous at the genital aperture with the internal wall of the genital ducts; and it is also continuous with the cuticular wall of the proboscides, and with that of the proboscis sheaths. It is a firm, uniform, structureless layer of variable thickness but never more than about 0·01 mm. in depth. It is said to be perforated by delicate canals running at right angles to its surface but I have been unable to see these. Normally it is finely granular in appearance and stains a light blue with methyl-blue-eosin. In sections which have been fixed with Zenker's fixative there is no appearance of lamination, nor of the deposition of the cuticle in any definite order; but in sections fixed in vom Rath's fluid and stained with iron haematoxylin I have observed a vertical striation. This is figured very accurately by Pintner (1880, Pl. V, figs. 3 and 4). I have no doubt that the appearance is that of an artifact produced by the action of the fixing reagent.

The cuticula rests on a layer which is usually called a basement membrane—but which appears to be only a specialised part of the underlying parenchyma. It stains pure blue with methyl-blue-eosin and is, as a rule, strongly contrasted with the adjacent layer of parenchyma, which stains with difficulty, and with the red staining integumentary muscles. Sometimes the basement membrane is so faint as to be unrecognisable.

The parenchyma. This tissue appears at first sight to consist of polyhedral cells which are closely apposed and possess walls which stain with difficulty, and cell bodies which do not stain at all, or only extremely faintly. It is usually so figured and it is so represented in Pl. XXIII, figs. 19 and 21, but it is certain that it consists of a relatively coarse reticulum which can be stained with methyl-blue-eosin by differentiating so that the red of the stain disappears and only the blue is left. The reticulum bounds spaces which contain only fluid contents. It includes small nuclei but it is difficult to be sure that these have any constant situation with regard to the meshwork. Among the other elements of the parenchyma are round or ovoid cells with prominent nuclei and these cells are large in comparison with the intra-reticular spaces: they probably belong to the same category as that to which the subcuticular cells belong. The parenchyma is the ground tissue of the Cestode, and fills the interspaces between the other organs. It can be traced right up to the cuticle, being differentiated beneath the latter to form the basement membrane.

The subcuticular cells. Pl. XXIII, figs. 19 and 21. These are situated beneath the basement membrane and form a more or less continuous layer embedded in the parenchyma. They are never so numerous as to form a closely packed stratum, and there is little significant difference in their relative abundance in different parts of the body. They are about 8 μ in longest diameter and have round or ovoid cell bodies which are produced into delicate prolongations which can be traced towards the cuticle: usually the subcuticular cells are so arranged that their long axes are perpendicular to the surface of the body. The cells have large nuclei, prominent nucleoli and granular cell protoplasm. Among them are apparently isolated nuclei which probably belong to the parenchyma.

The function usually ascribed to the subcuticular cells is that of the secretion of the cuticle. The latter appears to be a secretion from some underlying tissue; and since the subcuticular cells have a glandular appearance and send prolongations towards the cuticle just as unicellular cells are situated with regard to the surface on which they open, they have had this function ascribed to them. If we study the development of the genital ducts we find that these are at first apparently epithelial in nature, that is they have walls which are composed of two layers of rounded cells lying in close contact with each other. By-and-by a homogeneous membrane appears between these two layers of cells, and the latter then begin to degenerate, so that the wall of the duct finally becomes a membrane of homogeneous structure, and with cell nuclei on its outer and inner surfaces. It is supposed that the subcuticular cells are related to the cuticle in much the same way as the pseudo-epithelial cells are related to the developing genital ducts. But there does not appear to be any direct evidence in favour of either hypothesis; and it seems quite as likely that the wall of the duct is laid down as a secretion from the surrounding parenchyma, and that the cells, or nuclei, are functional in subserving the nutrition of the duct wall. If this is the case we can hardly regard the subcuticular cells as directly concerned with the deposition of the cuticle, for they bear a very similar relation to the latter structure. What then is their function? Pratt (1909) regards them as indifferent cells, or embryonic residual tissue, which develops into other specialised structures as the Cestode grows in size; or perhaps as glandular cells which secrete some substance acting as a means of defence against the digestive juices of the alimentary canal of the host. Now some such means of defence must, it appears to me, be postulated in the case of a parasitic Trematode or a Cestode but though the view is a probable one it cannot be backed up by direct evidence. Against it is the appearance of the cells, which is not a glandular one; and it seems to me that it is more likely that they are trophic in function and have to do with the nutrition of the cuticula.

The integumentary muscles. These are present as two main series and are represented in Pl. XXIII, figs. 19 to 21. Fig. 20 depicts part of a section made tangentially to the surface of the integument: it passes through the latter just beneath the cuticle and is thick enough to include both the longitudinal and transverse muscle layers. The transverse fibres are situated externally just beneath the basement membrane and are arranged as an uniform layer of very fine fibres. The underlying longitudinal fibres are much coarser and they run in irregular bundles enclosing interspaces. In the region of the scolex and in the more immature proglottides they form a very uniform layer, but in the more mature segments they are arranged in irregular bundles as is shown in Fig. 21, the bundles being elongated at right angles to the integument.

In the region of the scolex a second series of transversely running fibres may be present just internal to the longitudinal layer: these are shown in Pl. XXIII, fig. 19. They are quite absent in the mature proglottides. In the scolex other muscle fibres may also be present; some of these run dorso-ventrally, or at right angles to the axis of the scolex in various planes; and some are evidently the prolongations of myoblast. But the true integumentary muscles consist of smooth unstriated fibres without cell bodies or nuclei. They always stain a bright red with methyl-blue-eosin.

The integument as above described varies only to a slight extent in different parts of the body, the chief modifications being those of the arrangement of the muscle layers.

VI. *The immature proglottis.*

The male genital organs. The following account of the anatomy of the proglottis, which I hope will add materially to our knowledge of the morphology of the Tetrarhynchids, is based almost entirely on the study of serial sections. Stained and cleared preparations of the whole proglottis help us very little in this respect, for the segment is nearly cylindrical in shape and can be flattened out to a very slight extent. The larger genital organs can indeed be made out but it is quite impossible to attempt to trace the relations of the ducts. The reconstruction of the organs from a complete series of sections is therefore the only practicable means of studying the anatomy of the proglottis.

Pl. XXI, fig. 10 is a reconstruction of the genital organs present in the immature proglottis and has been reconstructed from a complete series of transverse sections: it should be compared with Text-fig. 8, which represents a transverse section through a proglottis in the same phase. The former figure is slightly diagrammatic, that is the coils of the vas deferens and uterus are made less complex than they ought to be and the vitellaria are drawn as if they were two bands along the lateral margins of the segment: in reality they are present all round the periphery of the latter. The ovary is situated at the posterior end of the proglottis and the genital aperture is at the postero-lateral margin. From this common genital aperture two ducts proceed: one, the copulatory passage, or vagina, runs backwards with few convolutions and ends in a dilatation—the receptaculum seminis. The oviduct takes origin from the posterior margin of the ovary and is almost immediately joined by a very narrow duct from the receptaculum seminis—the canalis seminis. The oviduct now turns forward and is joined by the vitelline duct and it also receives the efferent stalks of the cells of the shell gland. The compound duct so formed, which may be called the uterine canal, runs forward being thrown into numerous convolutions and opens into a thick walled sac which lies along the ventral side of the segment. This is the uterus. It does not open to the exterior.

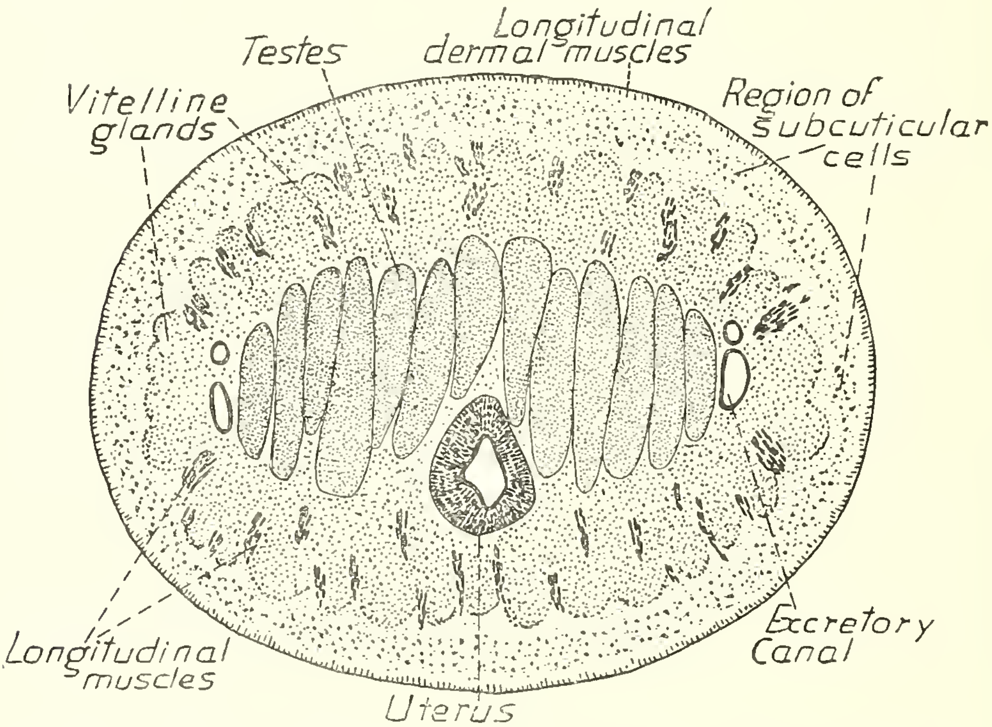

Fig. 8. Transverse section through an immature proglottis in much the same stage of maturity as that represented in Pl. XXI, fig. 10. The shading of the various organs is quite diagrammatic but their positions and proportions are fairly accurate. Mag. 70 dia.

The male genital tract begins as the cirrus—an evaginable tube surrounded by a muscular apparatus—the cirrus sac. Leaving the cirrus sac the male duct expands to form the vesicula seminalis and is then continued as a wide duct—the vas deferens, which runs forward in close coils, and then branches to form the vasa efferentia each of which finally ends in a testicular follicle.

We may now consider these various organs in greater detail.

The testes. I have called the proglottis represented in Pl. XXI, fig. 10 an immature one in relation to the development of the fertilised ova, but it really represents the maximum degree of development of the male organs. As the segment grows in size the male apparatus degenerates and only then do the female organs attain their greatest development. In the phase under consideration there are about 300–400 testes in a single segment, and they occupy a considerable proportion of the bulk of the latter. It is difficult to count their exact number or to represent them all accurately in one drawing as they are partially superposed on each other. Each is a short cylinder tapering towards one end (Text-fig. 8) and placed with its long axis in a dorso-ventral direction. They lie in the central part of the segment but there are always a number at the anterior end in front of the uterus. They are completely surrounded by the vitelline glands and dorsal and ventral to them are the longitudinal muscles of the proglottis. Each follicle is about 0·2 mm. in length, and about 0·05 mm. in diameter.

The testicular follicles in the youngest segments in which they can be identified are about 0·02 mm. in diameter. Each consists of a mass of undifferentiated cells, and there are usually about ten to twenty of these in a transverse section. There is no testicular membrane in these youngest testes, but as development proceeds a very delicate limiting membrane makes its appearance. The cells in the interior now become more numerous, the follicle grows, and differentiation begins. Pl. XXII, fig. 18 represents an immature testicular follicle and we see that there is an outer epithelial layer consisting of rounded cells which are not in close contact with each other, while within this are two kinds of cells. One kind, γ, resembles those of the periphery and are still undifferentiated; but the others, β, are evidently actively dividing. I have been unable to see any actual division figures but the material at my disposal was not such as would show these easily. These dividing nuclei are larger than the other cells and have very prominent chromatic synapses.

Pl. XXII, fig. 15 represents an older follicle from a riper proglottis and is cut longitudinally. There is now a very evident outer limiting membrane and the peripheral layer of cells has broken up, though undifferentiated cells of the same kind are to be seen in groups round the margin: these cells are about 4 μ in diameter and they always lie in groups against the outer membrane. At β can be seen cells similar to those so lettered in fig. 18 and like the latter they are large and have prominent chromatic reticula. They usually lie separately from each other. Other cells in the interior of the follicle are entirely undifferentiated, are small and stain faintly: they are evidently cells which are taking no active part in actual spermatogenesis.

The distinctive character of the follicle at this stage is the presence of relatively large capsular structures about 0·02 mm. in diameter (δ) consisting of an outer single layer of cells similar in character to those lettered γ. These cells are always arranged loosely and are hardly in actual contact with each other, so that it is sometimes difficult to be sure that they form an actual capsule and are not simply the limits of a space formed among the indifferent cells of the follicle. Within this capsule is an ovoid body which is about 0·016 to 0·018 mm. in diameter, and which is separated from the capsule by a space. It is coarsely granular in structure and has small projections on its periphery.

The above structures are all that are present in the maturing follicle.

All the follicles in the testis represented by Pl. XXI, fig. 10 are ripe and contain fully formed spermatozoa. They are now larger and looser in texture than in preceding stages. Pl. XXII, fig. 13 represents such a ripe testis cut longitudinally and it will be seen that considerable changes have taken place. The outer limiting membrane still exists but the layer of marginal cells has completely disappeared. A number of the small indifferent cells (γ) are scattered throughout the follicle and are embedded in a granular debris produced by the breakdown of some of the tissue elements. The large ovoid capsular structures still exist but they are undergoing degeneration, for the cells composing their walls are now further apart as if the structure were breaking up. The morula-like structure in the centre is much smaller, is homogeneous in nature and the space between it and the outer irregular layer of cells is filled with granular debris. The characteristic feature of this phase is the presence of large bundles of fully formed spermatozoa.

Spermatogenesis. It would be unprofitable in the meantime to discuss these appearances in the light of already published accounts of spermatogenesis in other Cestodes—before doing so a greater number of proglottides would have to be examined after fixing and staining by a variety of methods. But it is probable that the course of the formation of spermatozoa is much as follows:—the undifferentiated cells which are arranged round the periphery of the follicles in the youngest stages proliferate into the interior and give rise to two kinds of cells, the small indifferent cells, (γ), and the large nuclei (β). The latter divide actively forming the morula-like structures, which then become surrounded by the small indifferent cells so as to appear to be enclosed in capsules. The morula-like structures contain one or more sustentacular cells at their centres and round these are a great number of cells modified so that they come to consist only of the chromatic matter of the nucleus. The sperms are developed from these small nuclei for we find bundles of them in the later stages of the growth of the follicle still adherent to the sustentacular cells. But I have not actually seen the growth of the sperms themselves and it is possible that there may be other stages. The mature follicles contain only the bundles of sperms free or attached to the sustentacular bodies, the degenerate capsules and granular debris. The spermatozoa are of the usual type, but the heads are extremely small and cannot be resolved from the tail portions by a Zeiss apochromatic 1·5 immersion lens: their presence can only be deduced by a rather darker staining, and a blunter appearance at one end of the filament.

The vasa efferentia. These vessels are extremely delicate in structure, and as they do not appear to contain the sperms for any great length of time it is extremely difficult to trace them in the sections. They cannot be made out in stained and cleared preparations, but only in sections when several of them happen to lie in the same plane. They are very accurately figured by Lönnberg (1891). They can easily be studied in those parts of the proglottis where the typical parenchyma has almost entirely disappeared, and where the only structures present between the testes are the granular remains of the parenchyma, and the dorso-ventral muscle fibres. Their walls are very thin and stain with difficulty; when they are stained well they give the reaction of ordinary connective tissue substance. The wall however is a homogeneous layer which is continuous with the outer limiting membrane of the testicular follicle, and on its external surface are numerous nuclei which have prominent nucleoli and coarsely granular chromatic substance: it is remarkable that these nuclei cannot be seen on the limiting membrane of the testicular follicles, which nevertheless appear to be developed in the same manner as the walls of the vasa efferentia. There are no indications whatever in these structures of a wall composed of flattened epithelial cells such as is described by Erlanger (1890), while the vessels themselves are so distinct that the view of Moniez—that they are only hollowed out cavities in the parenchyma (1881)—seems to me to be highly unlikely. In the young proglottis the rudiments of the various organs first appear as cords or blocks of cells between which is the ground tissue which afterwards becomes the general parenchyma. This parenchyma secretes a homogeneous membrane round the various organs and ducts; and while the greater part of it must be used up, so to speak, in this process, the nuclei of some of the parenchymal cells remain and become applied to the structureless walls of the ducts and glands, where they probably exercise some nutritive function with regard to the latter.

The vas deferens. Vasa efferentia proceeding from the individual testes unite together to form vessels of the second and higher orders and these finally join to form a fairly wide duct which is the vas deferens. The vas deferens runs backwards from the anterior part of the proglottis, pursuing a highly convoluted course and finally opens into the genital pit. The structure of the wall is difficult to make out: it consists of an internal homogeneous membrane outside which are some fibrous elements and muscle tissue. The muscle fibres run for the most part longitudinally. There are numerous nuclei on the external surface of this wall and some of these evidently are of the same nature as those which are present on the walls of the vasa efferentia, but some of them may belong to the muscle fibres.

The vesicula seminalis. As it approaches the cirrus sac the vas deferens expands to form an ovoid body which is usually described as a vesicula seminalis: evidently it is only a dilatation of the vas deferens. The whole of the latter tract acts as a sperm reservoir, being usually densely packed with spermatozoa. The wall of the vesicula is much more muscular than that of the adjacent vessel, and perhaps the structure may function in some way with regard to the emission of the semen. From the vesicula a short, greatly narrowed tube pierces the wall of the cirrus sac.

The cirrus. The male duct, after it leaves the vesicula, is known as the cirrus, or intromittent organ. This part of the duct lies coiled up within the cirrus sac in three or four convolutions. Its wall differs in some respects from that of the vas deferens: the muscle fibres are more strongly developed and the longitudinal series is more pronounced than the transverse one. The cuticula covering the general surface of the body becomes reflected at the genital aperture and lines the internal surface of the cirrus, being thick near the terminal part, but becoming thinner and vacuolised as the cirrus passes into the vesicula seminalis. This cuticular lining is continuous with the homogeneous "basement membrane" of the vas deferens.

The cirrus sac. This structure (Text-fig. 9) is ovoid in shape and surrounds the cirrus and the terminal part of the vagina. Its wall is composed of dense parenchymatous tissue, and of fibrous and muscular elements, and is not definitely circumscribed but fades out into the surrounding parenchyma. Most of the muscle fibres take origin in the integument round the genital aperture and run longitudinally in relation to the long axis of the sac, but some of them do not run completely round the latter but pass out into the parenchyma, or are even recurved and are again inserted into the integument at a little distance away from the genital aperture. There are also transverse fibres which run completely round the sac, and these are strongly developed. In addition to these two series of fibres there are radial ones which originate in the outer wall of the cirrus and are inserted into dense parenchyma in the wall of the sac. Round the base of the genital papilla there is a strong development of the integumentary muscles so that a sort of sphincter is formed, and in this region the parenchyma is very dense. The longitudinal and transverse fibres appear to be nucleated but the cell protoplasm of these myoblasts is not very evident.

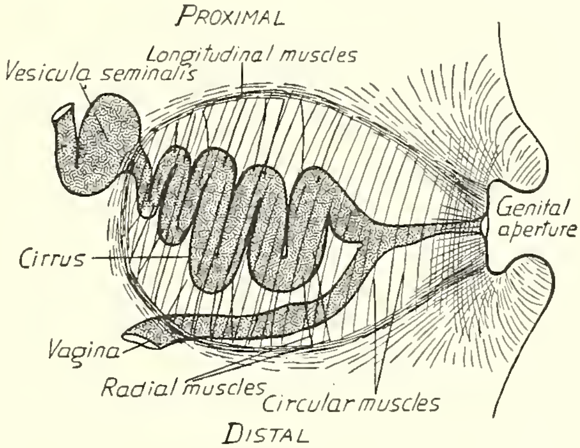

Fig. 9. Diagrammatic horizontal section through a ripe proglottis in the region of the cirrus sac. The arrangement of the muscles is schematic. Mag. 100 dia.

The genital papilla is a raised part of the integument and subjacent tissues surrounding the opening of the genital duct. The latter does not open directly on to the summit of this papilla, but into a shallow pit. The terminal part of the genital duct is common to both male and female tracts, being formed by the junction of the cirrus and the vagina, and it is apparently only this common genital duct that can be everted. Evagination of the cirrus is produced by the pressure on the duct of the fluid contained in the cirrus sac, so that the floor of the genital pit and the terminal part of the cirrus are turned out in just the same way as the proboscides are evaginated. Retraction of the cirrus is probably brought about by the relaxation of the walls of the cirrus sac, and the contraction of the radial muscles which then pull the cirrus inwards. I have only seen the cirrus everted on one occasion in a living proglottis, when it was situated on the summit of the genital papilla, the genital pit being itself then evaginated. It was about 1 mm. in length, and there were no hooks or armature of any kind, the outer surface of the wall being formed of a clear cuticular substance.

Since it is the common duct formed by the union of the terminal parts of the vas deferens and vagina which is evaginated, it does not appear to be possible that self-copulation can take place in *Tetrarhynchus erinaceus.*

The female organs. The female genital organs in *Tetrarhynchus erinaceus* conform to the usual Cestode type. The vagina passes back from the genital aperture and joins the oviduct immediately after the latter has issued from the ovary. The vessel so formed receives the vitelline duct and the efferent parts of the cells composing the shell gland, and then runs forward to form the uterus. It will be seen from Pl. XXI, fig. 10 and Pl. XXIV, fig. 11 that the greater part of the immature proglottis anterior to the genital aperture is taken up by the testes, the vas deferens and the vitelline glands; while the corresponding region of the mature proglottis is occupied by the pregnant uterus. The other organs are therefore restricted to the region behind the genital aperture and so great is the crowding here that it is quite impossible to make out the relations of the ducts in cleared preparations; and even in serial sections the junction of the various ducts is contained within comparatively few sections so that the reconstruction is a matter of some difficulty. I have simplified the actual conditions in figs. 10 and 11 by making the ducts a little larger than they ought to be and smoothing out the coils to some extent, otherwise the relations would be exceedingly difficult to represent in a diagram. In Pl. XXII, fig. 12, however, the various organs are drawn to scale.

The ovary. This structure (Pl. XXII, fig. 12; and Pls. XXI and XXIV, figs. 10 and 11) is bi-lobed consisting of right and left halves joined by a median bridge. It is secondarily lobulated and rather diffuse in structure, extending to some extent between the vitelline glands and other organs. The lobules are invested by a very delicate membrane apparently devoid of nuclei. The structure of the ovary is rather loose at the periphery, but much more compact in the region of the median bridge.

The ova (Pl. XXII, fig. 14) are polyhedral in shape towards the periphery of the ovary, but are quite spherical in the median parts. Their average diameter is about 0·01 mm. There is no distinct cell membrane. The nucleus has a very distinct membrane; the linin substance stains very faintly; and the chromatin is present as a synapse which exhibits a radiating arrangement of the threads. The nucleolus is very prominent and sometimes contains a round faintly staining body, sometimes one or two strongly refringent granules. In the cell substance, at the opposite pole of the egg to that towards which the nucleolus is situated, there is a "Nebenkern" in the form of a small round lightly staining body just outside the nuclear membrane. I have seen no signs of dividing cells in the ovary; probably this division occurs at a very early stage.

The oviduct. This takes origin at the posterior side of the median bridge, and here is situated the peculiar structure called the "Schluckapparat" by Pintner (1889), and the "Sphincter ovaricus" by Monticelli. It is a modification of the membranous wall of the ovary, which here becomes greatly thickened, and encloses a lumen which is about twice the diameter of a single ovum. Outside this there are a number of fibres arranged to form a thick ring, and running for the most part circularly, though some of them are also radial and run out into the surrounding tissues. These fibres have been described as muscular, though I suspect, from their reaction towards methyl-blue-eosin, that they are rather of the nature of elastic tissue. The whole structure only occupies some half dozen thin sections and then the diameter of the oviduct contracts till it is too narrow to admit of the passage of a single ovum. The "Schluckapparat" is described as able to execute rhythmic movements, expanding to allow an ovum to pass out from the ovary, and then contracting so as to force it into the oviduct. I suspect, however, that it is only an elastic sphincter, and that the expulsion of the eggs from the ovary may be brought about by the contraction of the muscles of the proglottis.

The oviduct enlarges considerably in calibre beyond the "Schluckapparat." Its wall here consists of an inner single layer of rounded cells rather flattened against each other in the manner of a true epithelium; of a basement membrane; and of an outer rather irregular layer of cells with very indistinct cell bodies. Further away from the ovary this outer layer of cells breaks down still more so that we have rather isolated nuclei embedded in a cell debris. The inner layer of cells also becomes very irregular and finally disappears so that the wall of the oviduct comes to consist only of the intermediate basement membrane. One is forced to regard this as the definitive wall of the duct, and the outer and inner layers of cells or nuclei as the formative organs of this structureless membrane. The canalis seminalis from the receptaculum seminis opens into the proximal part of the oviduct while it is still running posteriorly, and immediately afterwards the vitelline duct also so debouches. The oviduct now turns forwards, receiving as it does so the efferent stalks of the cells composing the shell gland.

The shell gland. (Pl. XX, fig. 10; Pl. XXIII, fig. 25.) This consists of a great number of flask-shaped cells arranged round the bend of the oviduct in the form of a hemispherical shell, the concavity of the shell being directed anteriorly. The cells radiate inwards towards the oviduct and join the latter near the place where the canalis seminalis and vitelline ducts open. Each cell, which is about 0·03 mm. in greatest length, is a unicellular gland, and contains a large nucleus in which there is a nucleolus with refringent granules. The distal part of the cell, which is bulb-shaped and contains the nucleus, stains light red with methyl-blue-eosin, but the proximal part is stalk-shaped and stains deep blue. The stalk runs towards the oviduct and then frays out into a number of delicate filaments which penetrate between the layer of cells, or nuclei, forming the outer portion of the wall of the former. At this part of the oviduct the basement membrane disappears and the filaments can be traced between the cells forming the internal layer, and can sometimes be seen even in the lumen of the duct. But it is probably the secretion of the cells which one sees here, for both the lumen of the duct and the interstices of its wall may exhibit the blue staining reaction characteristic of the secreting part of the shell gland cells.

The uterine canal. (Pls. XXI and XXIV.) We can recognise three regions in the female genital tract: (1) from the ovary to the junction of the canalis seminalis; (2) from the latter point to the opening of the vitelline duct; and (3) the remainder of the canal. Two views may be held with regard to the nature of the whole canal: (1) that it is the continuation of the vagina, and that both oviduct and vitelline duct open into it; (2) that it is everywhere morphologically a true oviduct which receives the canalis seminalis and vitelline ducts. However this may be it is evident that the canal beyond the place of entrance of the other ducts is different from the more proximal tract in that it contains eggs which are fertilised and yolked, and it has therefore been called by

Leuckart the "Befruchtungscanal." In *Tetrarhynchus* and some other Cestodes this canal opens into a wide sac—the uterus—but in such more primitive forms as *Bothriocephalus* the uterus begins immediately behind the shell gland, and there is no distinction between uterine canal and the uterus proper. Probably we may regard the differentiation into a narrow uterine canal and a relatively capacious uterine sac as dependent on the shape, size, and number of the proglottides. In such forms as *Bothriocephalus* there are a great number of segments and these are relatively small; whereas in the Tetrarhynchids the number of proglottides is small but the individual segments are, relatively to the strobila, much larger than in a *Bothriocephalus*, and are elongated in the longitudinal direction; while the ovary and other organs are concentrated in the posterior part of the segment. The whole female tract is probably morphologically one, but the uterine part becomes greatly expanded in the anterior part of the proglottis as the testes degenerate; while the posterior tracts must remain narrow because of the crowding of the other organs. It is purely for convenience that we speak of the posterior convoluted section of the female tract as the uterine canal, and the anterior section as the uterus.

The uterine tract, then, may be said to begin immediately after the opening of the vitelline duct. Its wall here has a very similar structure to that of the proximal part of the oviduct; that is it has a basement membrane with irregular layers of nuclei on inner and outer surfaces. Distal to the entrance of the vitelline duct the wall changes in character and consists solely of the structureless basement membrane, with some superadded fibrous tissue, and with adhering nuclei. The uterine canal is thrown into numerous convolutions which occupy the middle parts of the proglottis. Its most proximal part contains ova practically identical with those present in the ovary, and immediately beyond the opening of the vitelline duct these are mixed with yolk cells and these two kinds of cells lie together. Soon, however, the yolk cells break down, and the ova become yolked and acquire outer membranes.

The uterus. (Pls. XXI and XXIV, figs. 10 and 11.) The shape and dimensions of this structure are diagnostic of the stage of maturity of the proglottis; and it is represented in Pls. XXI and XXIV, figs. 10 and 11 in the immature and mature conditions. In the very youngest segments in which it can be recognised it is present as a simple cord of cells, which later on acquires a lumen; and still later it becomes a thick-walled tube (Text-fig. 8). So long as the testes are developing, or contain many spermatozoa, they occupy the greater part of the proglottis anterior to the genital aperture, and the uterus is then a straight tube of relatively narrow diameter, occupying the axial region of the segment. Presumably the testes are then in the actively functional stage, and copulation between the segment containing them in this phase and other segments may take place. But it seems to be quite probable that simple emission of the semen into the surrounding sea water may occur, and that the spermatozoa may be taken into the vagina from outside the segment. Self-fertilisation (but not self-copulation) may indeed occur but the mass of sperms contained in the testes in a ripe proglottis in the male phase is so great that it does not seem possible that all can be contained in the vagina. Some loss is therefore probable and it is likely that this occurs as the eggs ripen and are accumulated in the uterus.

In a younger stage than that represented in Fig. 8 the uterus is still smaller relatively to the other organs. It is roughly egg-shaped in transverse section, and has a restricted lumen and very thick walls (Pl. XXII, fig. 16). The basis of the latter is a structureless membrane of the usual type, inside which is a single layer of cells, and outside about four to six layers of cells. The basement membrane bears externally (in transverse section) a number of little projections which appear to be the cross sections of longitudinal fibres—these disappear in later stages. The internal nuclei are embedded in a matrix which appears to consist of broken down cell-bodies—certainly no cell outlines are recognisable and the surface facing the lumen is a broken and irregular one. The external cells are, on the contrary, very distinct: they are pear-shaped and the stalks run towards the basement membrane, though they cannot be traced into the latter. The nuclei are large and distinct and have prominent nucleoli. The whole wall has a kind of glandular appearance rather like that of the shell gland, but this appearance is illusory since the staining reactions of these cells are not, at any period, those of actively secreting structures. There can be little doubt that they are the formative blastema of the uterus itself, and that they become transformed into its structureless wall as the organ increases in size by the reception of the fertilised eggs.

The vagina. (Pls. XXI and XXIV.) This is a fairly wide and thin walled tube running back from the genital aperture. The latter it will be remembered is a wide, shallow pit situated on the postero-lateral margin of the proglottis. From the floor of this pit a canal takes origin—the common genital canal—and this soon branches, one branch becoming that part of the vas deferens which is situated within the cirrus sac, while the other branch penetrates the sac and passes backwards as the vagina. When the cirrus is evaginated it appears to proceed from the summit of the papilla—the genital pit being then turned inside out—and both vas deferens and vagina then open into everted cirrus. The wall of the vagina is thickest nearest to the genital opening and consists of a structureless membrane bearing nuclei on its outer surface with some muscle fibres running mostly in a longitudinal direction, though some are also transverse and oblique. The vagina runs back nearly in the middle line of the proglottis towards the space between the anterior lobes of the ovary—but it is not nearly so much convoluted as the uterine canal. Near the ovary and approximately in the mid line of the segment it enlarges considerably to form the receptaculum seminis. In the immature proglottis this dilated part of the vagina may be empty, but in segments approaching sexual maturity (in the female organs) all the canal, and particularly the dilated receptaculum, contain spermatozoa received from the same proglottis by self-fertilisation, or from another by copulation. The wall of the receptaculum is much thinner than that of the rest of the vaginal tract, being, of course, only a dilated part of the latter.

Near the median part of the ovary, and beyond the receptaculum, the vagina decreases considerably in diameter and its wall becomes much thicker. There is now a thick basement membrane with close, but rather irregular layers of nuclei on the inner and outer surfaces. The duct passes either over or under the median ovarian bridge and just posterior to the latter its calibre diminishes very suddenly and from its extreme posterior boundary a small tube takes origin. This is the canalis seminalis. It has a thick wall consisting of very dense fibres running circularly or obliquely. It runs very nearly at right angles to the former direction of the vagina and joins the oviduct very close to the junction of the latter with the vitelline duct. It is so difficult to be sure that it always enters the oviduct between the ovary and the junction of the vitelline duct that I am constrained to conclude that there may be some variation in the precise order in which these three ducts become connected with each other.

The vitellaria. These glands are represented in the diagram, Text-fig. 8, where they are shown in transverse section; and in Pls. XXI and XXIV, figs. 10–11, where they are represented only at the margins of the proglottis. They form a layer which is roughly concentric to the integument; and which is interrupted only at the region immediately round the genital papilla and lies between the layer of subcuticular cells and the testicular follicles. In the youngest proglottides the vitellaria are diffuse—thus they are represented in Pl. XXIII, fig. 21, as groups of cells lying quite loosely in the parenchyma just internal to the subcuticular cells. In later stages, however, they become compact and in Pl. XXII, fig. 12, which represents a part of a section of a mature segment, they are seen to be composed of distinct follicles. The vitelline glands originate in the very young segments as cords of cells, which later on acquire a very thin limiting membrane from the secretion of the adjacent parenchyma; and then become arranged to form short tubular follicles connected by duct-like structures. The terminal follicles are arranged radially so that they run out from the axis of the proglottis in all directions towards the periphery. The conducting portions of the follicles run towards the axis of the segment and join together to form longitudinal collecting ducts. But there is never any absolute distinction between the secreting and conducting parts of the gland except that the latter are generally a little less in diameter than the follicular parts. All parts are, however, filled with the yolk cells.

Pl. XXII, figs. 12 and 17, represent small parts of the vitelline gland under a higher magnification and it will be seen that the cells contained in the follicles in the earliest stages are surprisingly like the cells in the ovary, so much so that it is sometimes difficult to distinguish between the two kinds, especially as the limiting membranes of the two organs are so tenuous that it is occasionally difficult to trace them. Fig. 17 represents one of the vitelline follicles cut in longitudinal section. There is an outer limiting membrane and inside this there are usually two kinds of cells. One kind are small and resemble the ovarian cells except that the chromatic synapse is not so prominent. The other kind includes cells which are much larger, and are polyhedral in shape because of their mutual pressure against each other. They have large nuclei, faintly staining nucleoli, and the cell substance is almost entirely composed of coarse brightly staining granules—obviously yolk substance. In a section of one of these follicles there are usually about ten to twenty of these cells in a field. The small cells are similar to those in the peripheral parts of the very young proglottis: they are obviously still almost entirely undifferentiated.

Posterior to the ovary the collecting ducts of the vitelline glands run transversely to the long axis of the proglottis and then join together near the middle of the segment. These ducts are greatly enlarged and may be spoken of as a yolk reservoir. From this enlarged part a much narrower duct runs towards the uterine canal and opens into the latter just as it turns round to run forward.

The muscles of the proglottis. In addition to the muscles of the integument already described there are two series of longitudinal muscles and one series of dorso-ventral muscle fibres present in the segment. The longitudinal fibres are continuous with those which have already been described as originating in the proboscis bulbs. The latter fibres begin as four bundles which soon become gathered up so as to form two flattened sheets respectively dorsal and ventral in position and running roughly parallel to the dorsal and ventral surfaces of the proglottis. At first these sheets are almost continuous but they soon begin to be split up into distinct bundles. As the vitellaria and testes develop the bundles become relegated to their definitive positions and run longitudinally between the vitelline glands and testes—or even among the follicles of the former organs. Each bundle consists of comparatively few fibres and these are always dorsal or ventral to the testes and never run among the follicles of this organ. The lateral margins of the proglottis are marked by the presence of the main canals of the excretory organs, and just dorsal and ventral to each lateral pair of these canals there is a bundle of longitudinal muscle fibres which is larger than the others.

In the anterior parts of the strobila, where the segmentation is still incomplete, the longitudinal muscles run without interruption from the one proglottis to the next, but when the genital organs begin to be marked out this continuity of the longitudinal muscles ceases. Between every two adjacent segments there is a very narrow connecting region which seems to be composed of a tissue resembling dense parenchyma similar to that which is found in the integument just beneath the basement membrane—it is indeed continuous with this part of the integument. The ends of the longitudinal fibres are inserted into this hardened parenchyma, and the terminal parts of the fibres themselves become tendinous in nature. The longitudinal fibres forming the integumentary muscle system are also inserted into this connecting tissue and are not continuous from segment to segment.

The dorso-ventral muscle fibres are present in the young and mature proglottides but they appear to attain their fullest development in the latter: probably they become differentiated to the greatest extent as the proglottis becomes sexually ripe and acquires independent powers of locomotion. These fibres have been beautifully figured by Pintner (1880). They are smooth and band-shaped and the fibrous part runs apparently from the dorsal to the ventral surfaces of the proglottis. About midway on the course of each is the muscle cell, which has the form of a plate along one side of which the fibre is embedded. The cell has no membrane and is reticular in structure. The nucleus is large; has a prominent nucleolus; and a chromatic network restricted to the peripheral parts.

The excretory organs. I describe here the main canals only and not the finer terminations, nor the terminal flame cells. There are four main canals in each proglottis, two of which run longitudinally along each lateral margin. In transverse section one of these canals is always larger than the other, this one is the more ventral of the two. The canals are situated between the testicular follicles and the vitelline glands, but usually the presence of the canal, with the large bundles of longitudinal muscle fibres which are situated adjacent to them, causes the vitelline glands to be less abundant at the lateral margins of the segment. The canals are not straight but are usually loosely coiled—an appearance however which may be due to a longitudinal shortening of the proglottis after fixation, for when the Cestode is killed in fresh water and fixed in weak formalin the canals may be quite straight. This condition may however be produced by the absorption of water by the tissues of the worm, and perhaps the accumulation of this in the excretory system. As a rule the canals are compressed in transverse section and have walls which are rather thick, and of no recognisable structure; but sometimes the outline of the canal in transverse section may be perfectly circular and the wall itself may be exceedingly thin. These lateral canals, certainly the larger one of each side, run without interruption from one segment to the next, and at the junction of two adjacent proglottides there is an anastomosing transverse branch which connects the canals of the two sides. When the segments separate from each other the cleavage takes place along the middle of this transverse anastomosis, so that the canals then open to the exterior at each side of the segment. There are no terminal vesicles, nor valves at the origin of the transverse anastomoses.

VII. *The mature proglottis.*

It is quite unnecessary to describe in detail the anatomy of the ripe proglottis since the relations of the various organs are very much the same as in the immature segment. The differences consist of the progressive development of the uterine sac as the segment enlarges; and of the degeneration of the testes, the vasa efferentia, vas deferens, and vitellaria. Pl. XXIV is based on a reconstruction of a mature segment from serial sections: the segment was one which was still a part of the strobila. The proglottis still continues to increase in size after dehiscence from the strobila, and I have no doubt, though I have not made sections from such a free proglottis, that the progressive degeneration of all the organs other than the uterus still proceeds while the segment is free within the alimentary canal of the host, or even lying freely on the sea bottom.

The uterus is the seat of the principal changes accompanying maturation. Originally a cylindrical straight tube of no great diameter it continues to increase in diameter as it becomes filled with fertilised eggs, until it occupies by far the greater part of the volume of the proglottis in front of the genital papilla. Originally its walls consisted of a cuticular membrane lined internally with a single layer of nuclei, and externally with several layers of cells, but in the mature phase it becomes a very thin membrane which bears internally and externally widely separated nuclei. The expansion of the uterus is brought about by the formation of the lateral evaginations of its wall and then these flatten out, or fuse together. The contained embryos are about 0·04 mm. in diameter and each has a very distinct cell membrane and contains a small number of cells embedded in a granular matrix. The embryonic hooks are not present—that is the oncosphere stage is not attained while the embryos are still within the proglottis, at least not in the stage while the developing segment still forms part of the parent strobila.

A "birth-pore" is probably not formed in the proglottis of *Tetrarhynchus erinaceus* and the embryos are only liberated from the proglottis when the latter undergoes dissolution. Nevertheless a birth-pore may possibly be formed at a later stage than I have been able to study, for such an opening is certainly present in the proglottides of the allied species *Tetrarhynchus tetrabothrius.* When a ripe segment of the latter worm is observed closely—the strobila lying in fresh water—the extrusion of the eggs may be seen, especially if after immersion in fresh water the worm is put into weak formalin. The eggs appear to issue from the dorsal or ventral surface of the proglottis and sometimes it appears as if they came from the genital opening; but I suspect that in the latter case the opening breaks through near to the genital papilla. The birth-pore does not appear to be constant in position, and it is probably formed where the wall of the proglottis is thinnest: it is a purely secondary structure and not a true uterine pore. I would refer here to Braun (1894–1900, pp. 1438–1451) for a discussion of the morphological significance of this opening in Cestodes generally.

I describe very briefly the principal changes which take place as the proglottis passes from the immature to the sexually mature condition.

Testes and ducts. The testicular follicles degenerate completely in the ripe proglottis. In stages a little younger than that represented in Pl. XXIV the testes are restricted to a very narrow zone between the layer of vitellaria and the wall of the uterus, and even here they may be present as a layer of only one or two follicles in depth. Some are also present at the extremities of the segment. In older segments such as that represented in Pl. XXIV the marginal testes degenerate completely and there are only a few left at the ends of the proglottis. The few that remain at the anterior end of the segment degenerate later on, and in the oldest proglottides that I have sectioned there are only a few testes in the part of the segment behind the ovary. In these later stages, the testicular follicle becomes much smaller than in the early phases, and also becomes spherical in shape. The distal end of the vas deferens persists in the oldest segments that I have seen, but it becomes relatively short, the convolutions of the tube become smoothed out, and its calibre diminishes. Till the end, however, it still contains ripe spermatozoa.

The vagina. This canal is more voluminous and more richly convoluted in the ripe than in the unripe proglottides; and the proximal part described as the receptaculum seminis is greatly enlarged. Throughout its entire length the vagina is filled with spermatozoa in the later stages of the segment. Its relations to the ovary and oviduct are the same as in the earlier stages.

The vitellaria. These glands undergo great reduction as the segment enlarges. All those which were present in the peripheral regions of the proglottis external to the uterus practically disappear and only a few persist at the posterior end of the segment, lateral and posterior to the ovary, and the terminal parts of the ducts also persist here as the yolk reservoir. In front of the genital papilla the body wall becomes very thin and consists only of the integumentary tissues and some remains of the vitellaria. Dorsally and ventrally the wall is thinnest and it is here that a birth-pore would most easily be formed.

Further investigation is, of course, necessary in order that the complete life history of our Cestode may be elucidated. It is first of all essential that the true larval host should be found: this, as I have suggested above, is probably an invertebrate which is eaten both by the collateral hosts (*Trigla*, *Gadus* etc.) and by the final, or adult hosts (Skates and Rays). But the distribution of the Plerocercoid larvae in the collateral hosts is so much commoner than the distribution of the adult worm in the final hosts, that it seems very probable that the true larval host is an invertebrate, regularly eaten by Teleosts belonging to the genera mentioned, but only exceptionally eaten by Skates and Rays.

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EXPLANATION OF PLATES XIX—XXIV.

PLATE XIX. The strobila and scolex.

Fig. 1. The strobila: mag. 5 dia.

Fig. 2. The scolex seen from the side: mag. 30 dia.

Fig. 3. The scolex: nearly dorsal view: mag. 30 dia.

Fig. 4. Transverse section of the scolex passing through the posterior region of the bothridia. The proboscides are extruded: mag. 75 dia.

Fig. 5. Transverse section of the scolex passing through the neck at the region of the proboscis bulbs. Mag. 94 dia.

PLATE XX. The proboscis.

Fig. 6. The proboscis showing one row of hooks. Mag. 580 dia.

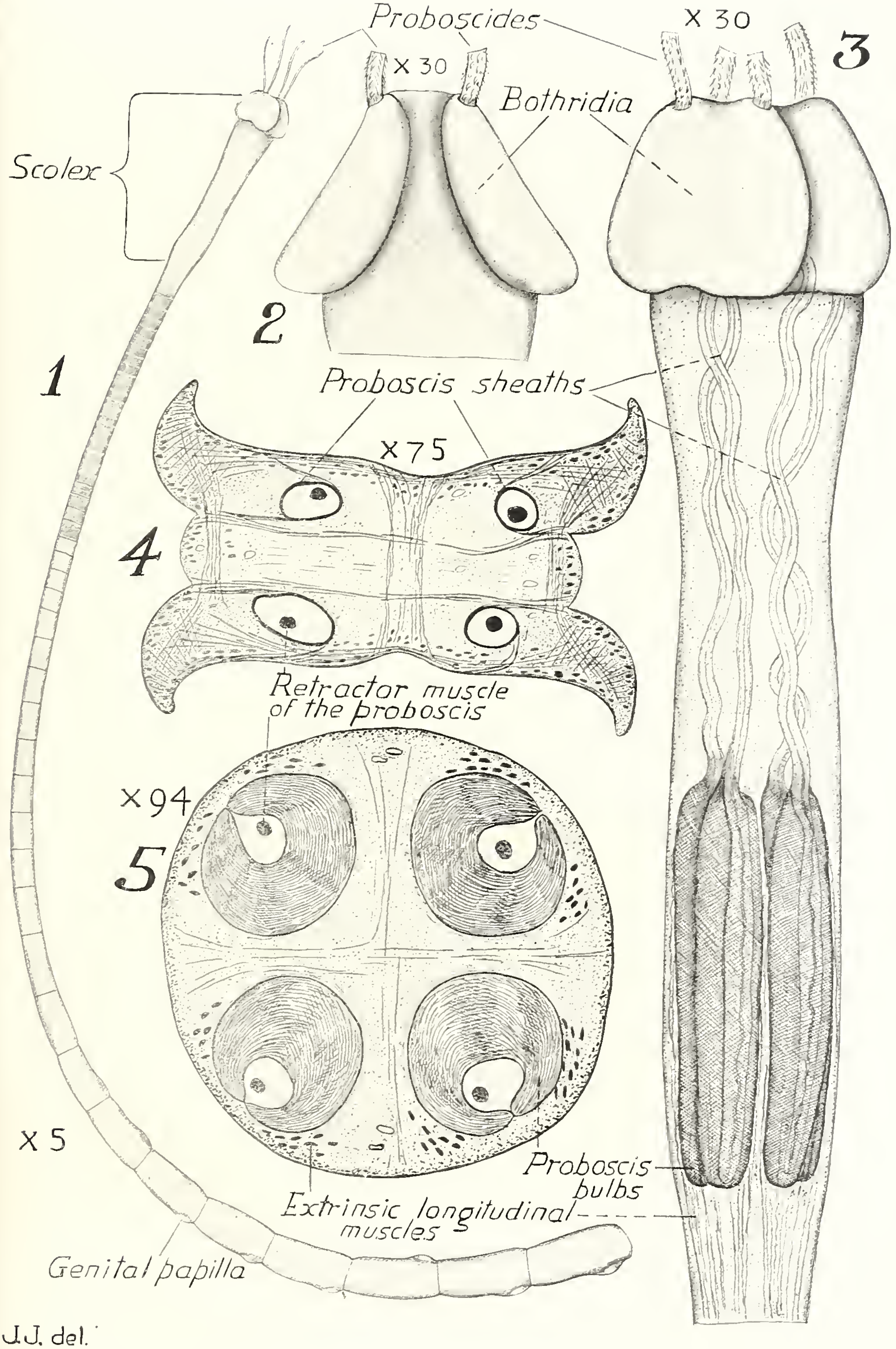
Fig. 7. The proboscis from the opposite side. Mag. 580 dia.

Fig. 8. One view of a proboscis taken from a plerocercoid worm encysted in the muscles of the halibut. Mag. 280 dia.

Fig. 9. *A*, *B*, *C*, *D*. The different hooks and spines on the proboscis of the adult worm. The numbers beneath the various figures are the actual sizes in millimetres of the hooks measured along the diameters represented by the lines and arrows.

PLATE XXI. The immature proglottis.

A semi-diagrammatic representation of the organs in the immature proglottis. The relative sizes of the various organs are as nearly as possible accurately represented, but the vasa deferentia and uterine canal are drawn with fewer convolutions than are actually present. The testicular follicles are represented as less in number than those actually present, and for simplicity's sake those overlying the uterus have been omitted. The vitellaria are represented as two marginal bands (but see Text-fig. 8). Neither the vasa efferentia nor the excretory canals are represented. The view may be either dorsal or ventral since the genital pit may be situated on either side of the proglottis. Mag. 65 dia.



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Tetrarhynchus erinaceus: strobila and scolex.

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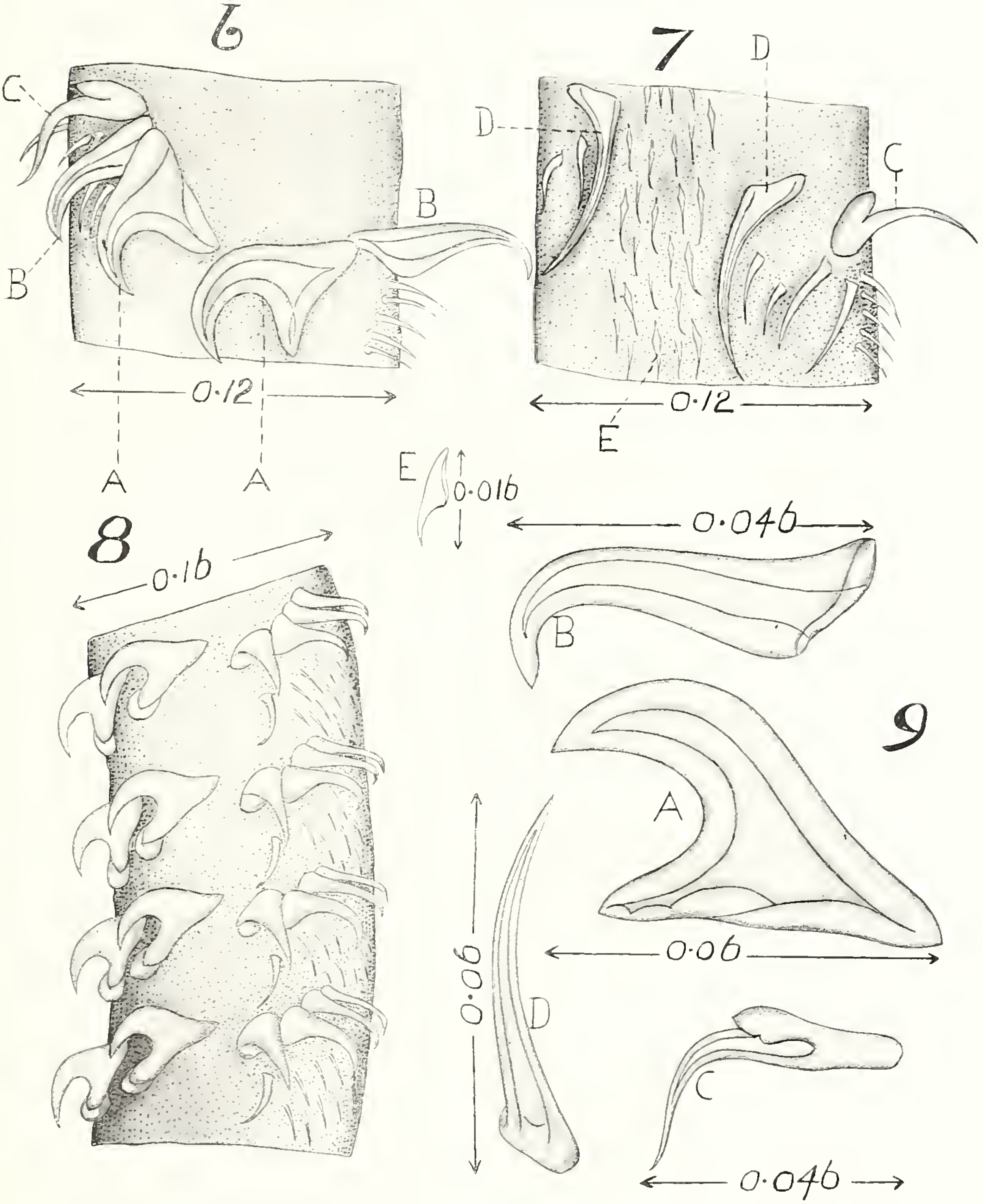

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Tetrarhynchus erinaceus: the proboscis.

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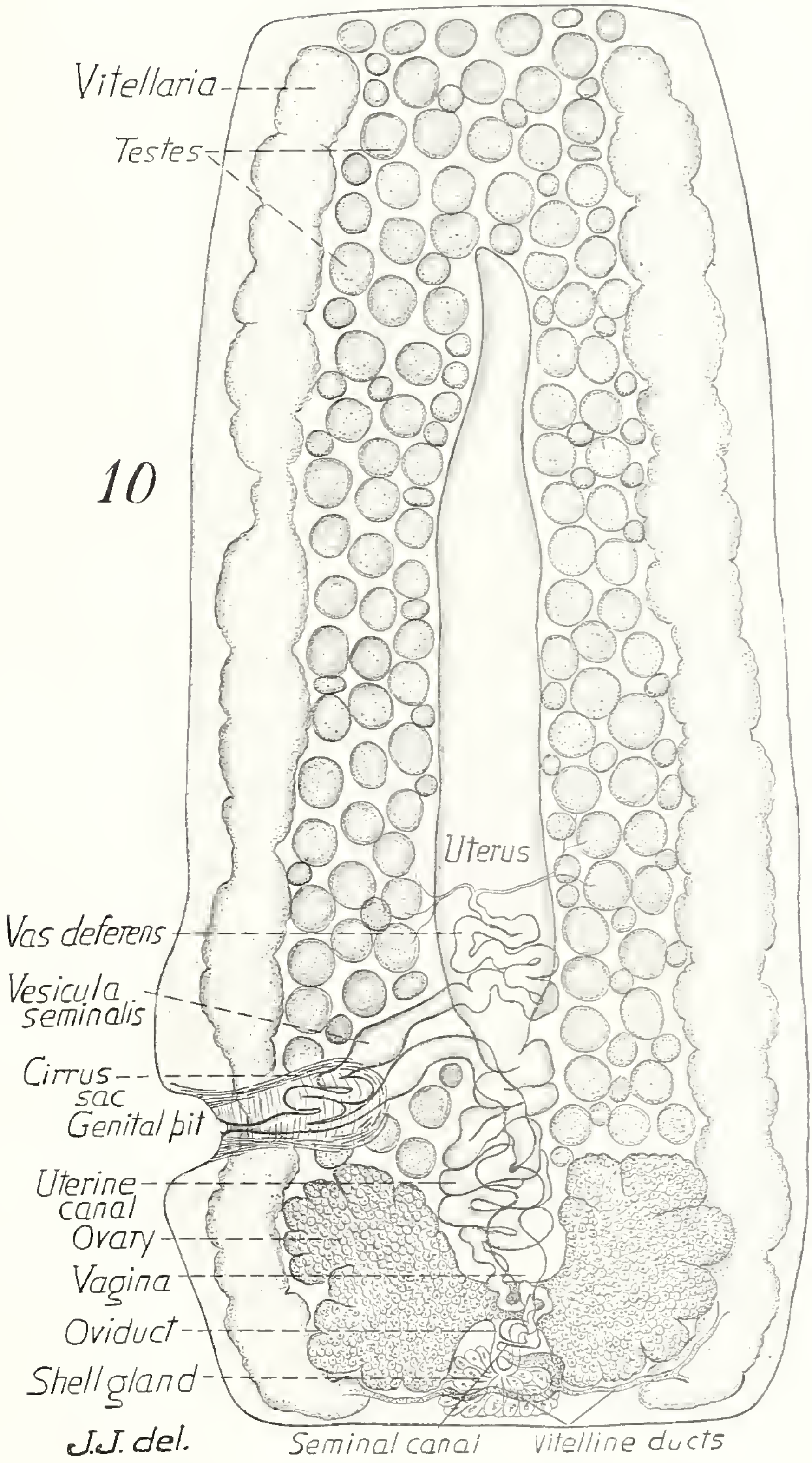

Tetrarhynchus erinaceus: the immature proglottis.

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NATURAL HISTORY

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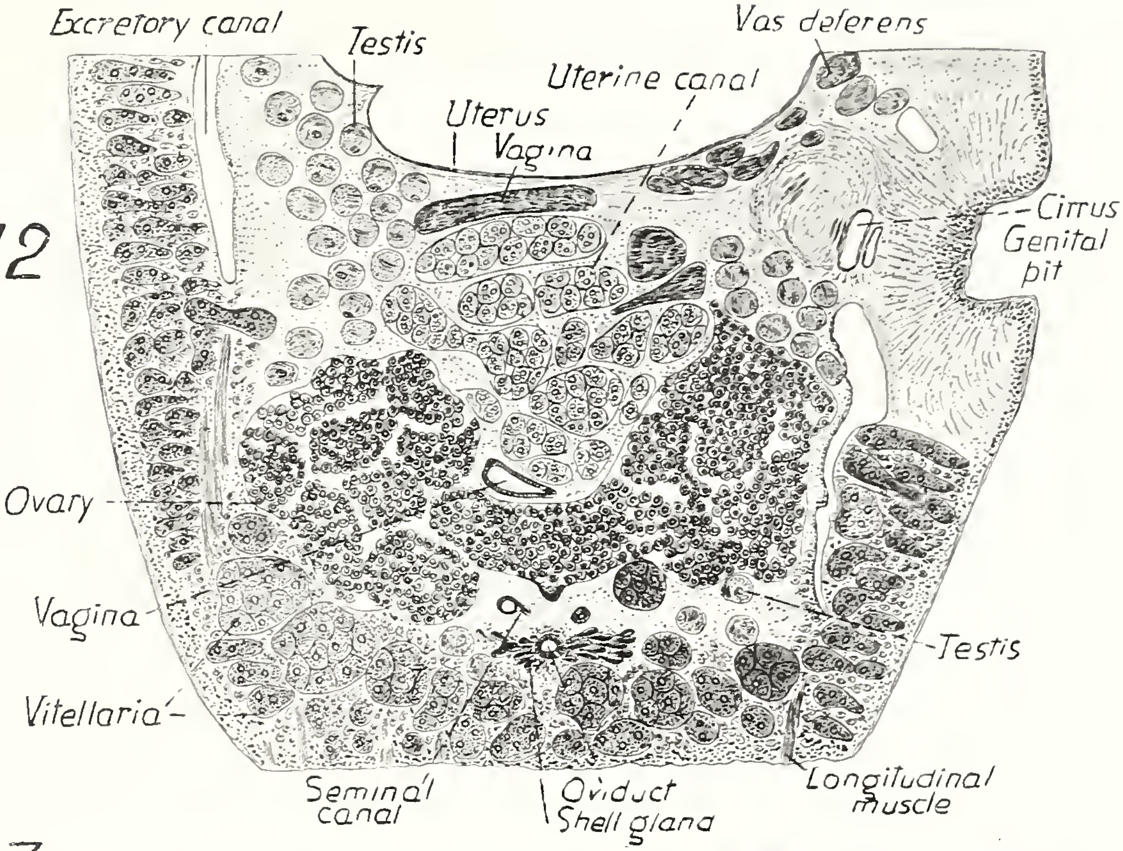

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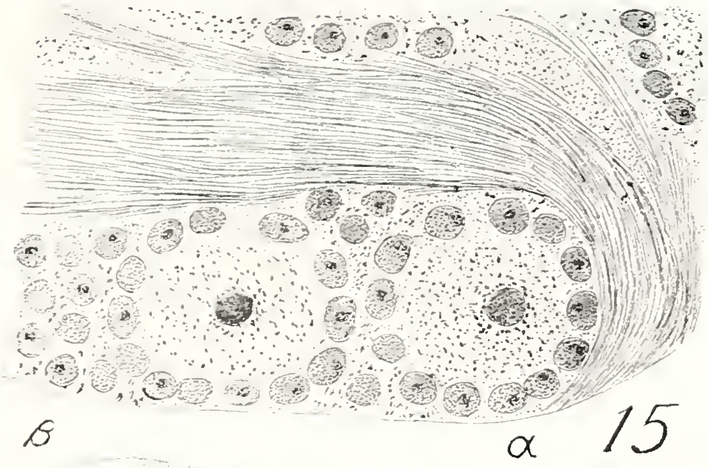

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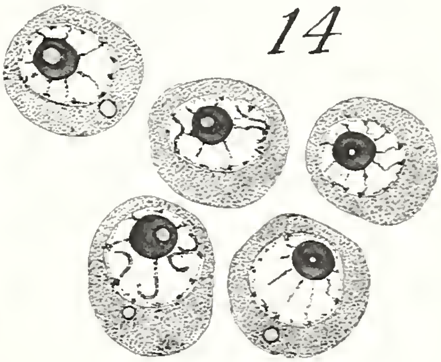

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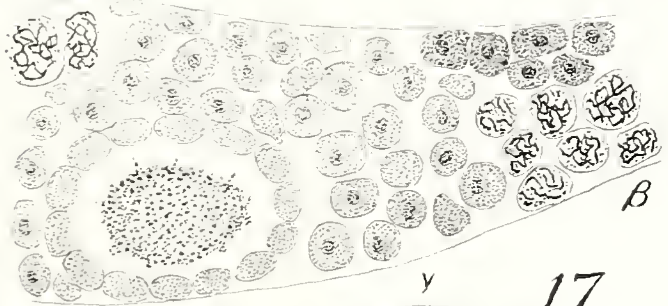

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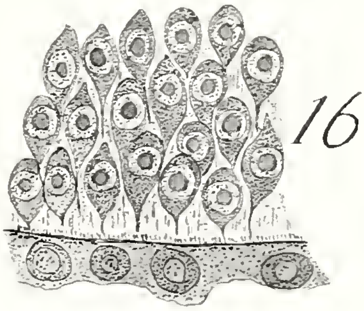

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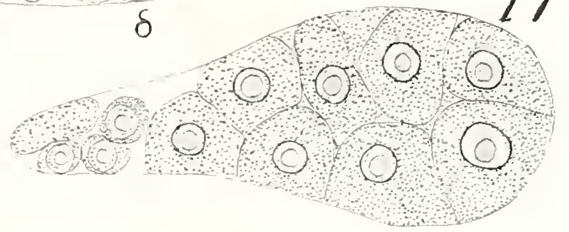

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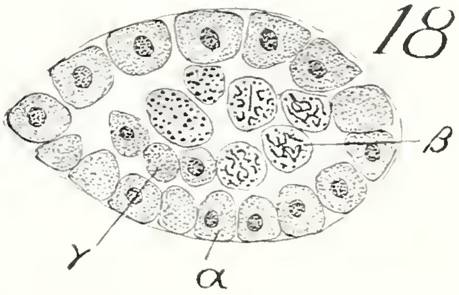

J. J. del. *Tetrarhynchus erinaceus*: genital organs.

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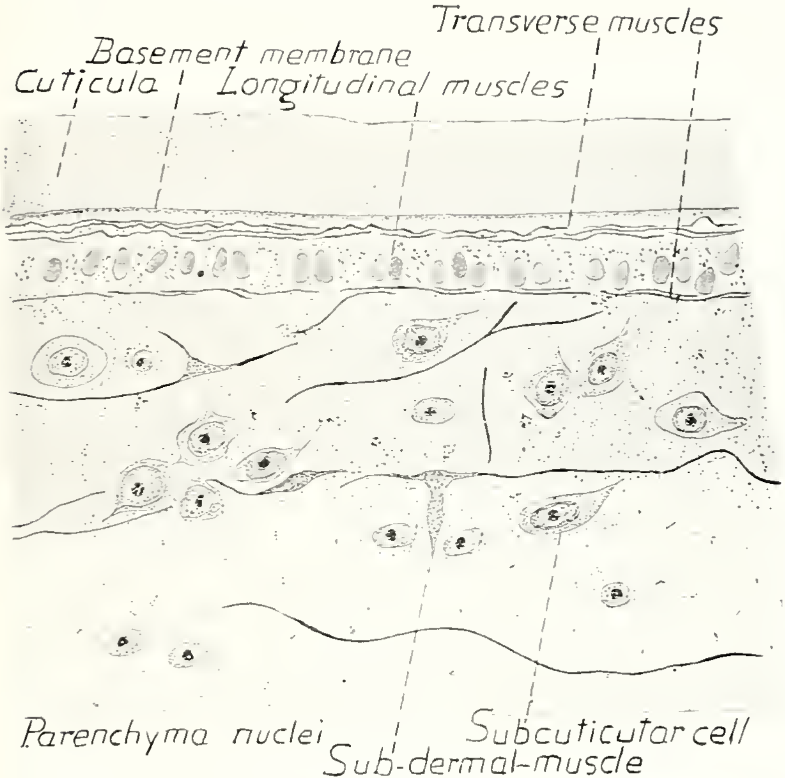

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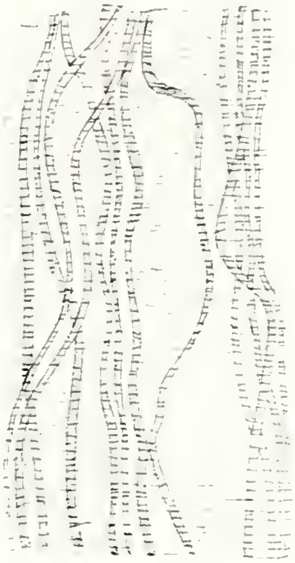

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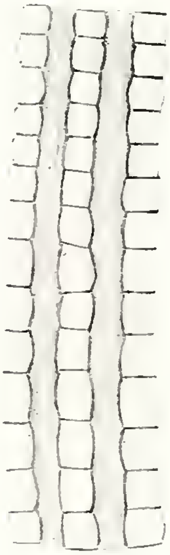

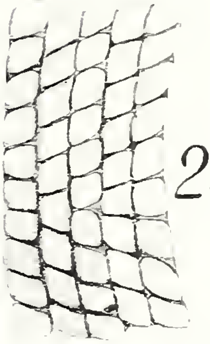

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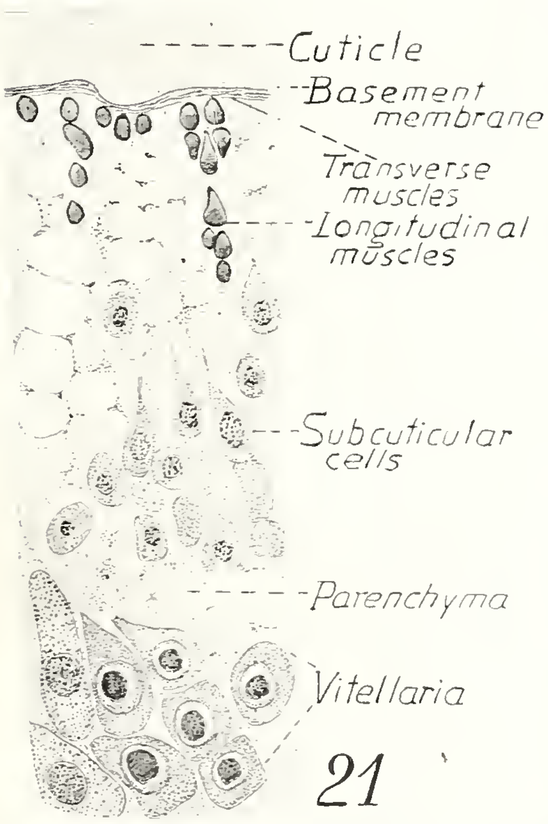

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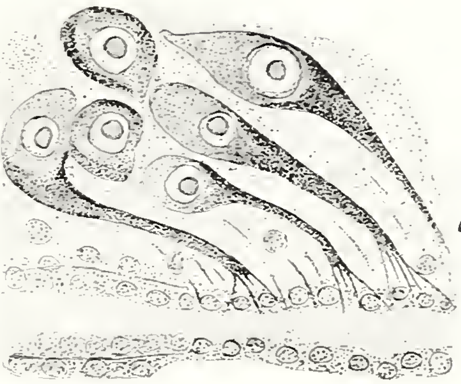

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J. J. del. *Tetrarhynchus erinaceus:* integument, musculature, etc.

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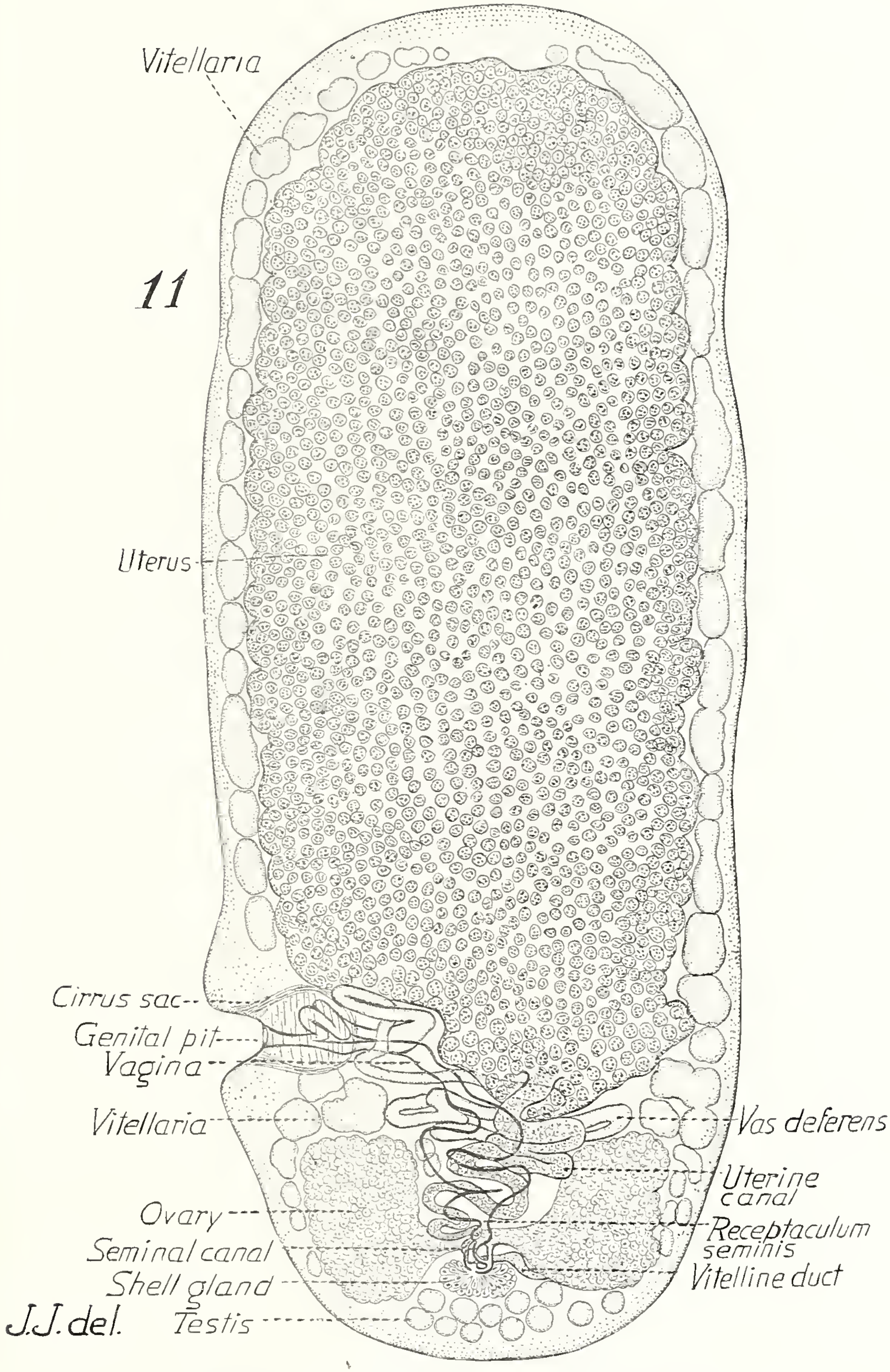

Tetrarhynchus erinaceus: the mature proglottis.

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PLATE XXII. The genital organs.

Fig. 12. Horizontal section of the part of the proglottis lying behind the genital papilla. The proglottis is nearly mature. Mag. 100 dia.

Fig. 13. A testicular follicle. The section passes nearly in the horizontal plane of the organ. The explanation of the Greek letters is given in the text at pp. 397, 398.

Fig. 14. Ovarian eggs. Mag. 1500 dia.

Fig. 15. A testicular follicle cut in the same plane as that represented in Fig. 13. The latter figure represents the more mature phase. Mag. 900 dia.

Fig. 16. Part of a transverse section of the wall of the uterus. The lower-side of the figure represents the surface which faces the lumen. Mag. 500 dia.

Fig. 17. Longitudinal section of a follicle of the vitelline gland. Mag. 500 dia.

Fig. 18. Transverse section of an immature testicular follicle. Mag. 900 dia.

PLATE XXIII. Integument, musculature, etc.

Fig. 19. Part of a transverse section of the integument and underlying tissues in the region of the least mature segments.

Fig. 20. Part of a tangential section through the integument. The section is thick enough to contain both the transverse and longitudinal integumentary muscle fibres. The thin horizontal lines represent the former, and the thick wavy lines the latter. Oil-immersion lens.

Fig. 21. Part of a transverse section through the integument and underlying tissues of a nearly mature segment.

Fig. 22. A small part of a transverse section through the musculature of a proboscis bulb. The latter is greatly contracted. The diameter of the lighter shaded "fibres" is about $2\,\mu$.

Fig. 23. A similar section from a proboscis bulb. The diameter of the "fibres" is about $3\,\mu$.

Fig. 24. Transverse section of the retractor muscle of the proboscis.

Fig. 25. Part of a transverse section through a mature proglottis showing the shell gland opening into the oviduct.

PLATE XXIV. The mature proglottis.

A semi-diagrammatic representation of the organs of a ripe proglottis. The relative positions and proportions of the various organs are as accurately preserved as possible; but, as in Pl. XXI the coils of the uterine canal and vas deferens are drawn more simply than is actually the case.

A REVIEW OF THE BRITISH MARINE CERCARIAE.

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(With Plates XXV—XXIX.)

General Life-History.

THE study of the life-histories of digenetic Trematodes is as yet in its infancy. Many new adult forms are described every year, but very little is known of the life-history of any of these. It is even impossible in the majority of cases to say what sort of larval form is characteristic of any group. Having worked for some years at the study of Trematode larvae I thought it would be of some use to bring together all the work so far done on British marine cercariae in order to form a nucleus round which future work may be centred.

As is to be expected more is known of the larval stages of bird Trematodes than of those inhabiting fish, as a great many sea birds are shore feeders and it is much easier to find cercariae in the shore animals than in those from the sea where the fish feed. It is only with fish and bird Trematodes that we deal in the present memoir.

From the fertilised digenetic Trematode egg issues a ciliated larva, the miracidium, possessing certain larval organs, which is free-swimming and after emerging from the egg in the water makes its way into some invertebrate host, the first host. This is in almost every case known a Mollusk, either Gastropod or Pelecypod. (The only exception known to me is that suggested by Pratt (1898, p. 351) who thinks it probable that a Hemiurid larva develops from a miracidium in a Crustacean host, namely a Copepod[1].) Having bored its way into this host the larva settles down in some organ, usually the digestive gland or gonad, loses its larval organs and degenerates into a sac, the sporocyst. From the cells of the walls of the sporocyst are budded off germ balls which usually grow into a different kind of larva, the redia, which bursts from the sporocyst and leads a free existence in the host. The sporocyst may however by budding proliferate without forming rediae and may form sporocysts within it instead of rediae. The sporocyst is merely a sac and has no alimentary canal, imbibing its food through the body wall; the redia on the contrary has a well-developed sac-like intestine and a mouth at the anterior end with a strongly muscular pharynx. It may also have a thickening round the anterior end of the body, the "collar," a birth-pore usually near the pharynx as exit for the cercariae, and at the posterior end two ambulatory projections which help it in progression. These latter organs may be entirely absent and frequently the ambulatory processes are only present in the young forms, probably because the redia migrates in its young state to some other organ of its host. Both sporocyst and redia may be coloured yellowish or a brilliant orange; the intestine of the redia is often brown.

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[1] The larva of the human Trematode *Bilharzia* is apparently capable of direct infection.

The redia, or, in those cases where the redia stage is omitted, the sporocyst now forms within it another kind of larva, the cercaria, which has the real Trematode form and gradually grows within its "nurse" from a shapeless mass of cells to a worm which shows more or less of the adult characteristics. Typically the cercaria has a tail and when it is full-grown it emerges from the redia or sporocyst. If the birth-pore is present in the redia the cercaria comes out in this way without rupturing the redia body wall, in the other case it bursts through the wall which closes up after it. All stages may be in the redia or sporocyst at the same time, from shapeless germ balls to the full-grown cercaria. The tailed cercaria now leaves its host altogether and swims about in the water for a little while. It then either comes to rest on some inanimate object (*e.g.* a blade of grass as in the Liver Fluke) and losing its tail forms round itself a cyst and rests, or it makes its way into another host, the second or intermediate host, and here it rests, usually forming round itself a cyst (in exceptional cases such as the "Pearl Trematode" a cyst is formed round the cercaria not by the worm itself but by the epithelium of its molluscan host). Up to a certain point the cercaria may feed and grow in its second host but afterwards it always has a real resting stage which is comparable to the pupal stage of a butterfly. The suckers, alimentary canal and excretory system are usually formed in the cercaria before it leaves its first host and parts of the reproductive system are sometimes developed. The alimentary canal however is not always formed so soon, especially in those forms in which extremely large unicellular glands occupy the greater part of the body. There are two kinds of glands which are sometimes very conspicuous in the cercaria, the cystogenous glands which secrete the material for the cyst and those glands which always are present if a boring spine or stylet occurs in the oral sucker. These latter glands are the "Stacheldrüsen" or stylet-glands which help the worm to bore into its host, the stylet piercing and the glands pouring out their fluid at the same time. Both cystogenous and stylet glands may be present or only one kind. The cystogenous glands sometimes occupy almost the whole of the body as in some of the Echinostomes, others occur in small masses; all open by ducts near the oral sucker. The stylet glands disappear after the cercaria has successfully entered its second host. The cystogenous glands disappear after the cyst is formed, as does also the tail. The intermediate host may be a Vertebrate or an Invertebrate. In marine Trematodes fish serve as Vertebrate intermediate hosts, the cercariae often encysting under the skin, in the muscles, nerves and various organs. Crustacea and Mollusca are the usual Invertebrate hosts; crabs and Amphipods are very commonly infected, the cercariae occurring in the liver, gonads or muscles. In Mollusca the foot, mantle, digestive gland or gonad serve the purpose. Nearly always the intermediate host, if a Mollusk, is a Pelecypod. In some cases the encysted stage takes place within the sporocyst. Here the life-history is much abbreviated and often the cercaria is tailed but loses its tail before it encysts. In other cases the resting stage is omitted because the final host swallows the redia with cercariae; here again we find the cercaria loses its tail before it is full grown.

In one group, the *Gymnophallus* group, the cercaria is always without a tail, and apparently has to reach its host by creeping movements or depend upon currents to sweep it along. This was noticed by Jameson (1902, p. 160), who found that because of the cercariae of the "Pearl Trematode" not having tails only those mussels fixed on the ground were much infected with pearls; those clinging to wood above were almost free from them.

Classification of Cercariae.

Lühe (1909, p. 173) has classified the various cercariae according to their tails but there appears to me to be something to be said against this method, excellent though it be in many ways. The chief objection seems to be that certain forms, which by their organisation are clearly very nearly related, are absolutely separated in this way, because in some of them owing to an abbreviation of the life-history the cercaria has lost its tail. In the *Spelotrema* group as described below we have a free-swimming tailed cercaria in *Spelotrema excellens,* cercariae such as *Cercaria oocysta* in which the young forms possess tails but do not use them (as encystment takes place in the sporocyst, where they are cast off), and finally in *Cercaria littorinae-rudis* we have most certainly one of the *Spelotrema* group which appears to lack a tail altogether. We are not justified in such a case, I think, in placing these cercariae in different divisions because of the presence or absence of a tail. Again *Acanthopsolus lageniformis* loses its tail before emerging from the redia. As a full-grown cercaria it is tail-less, but it is much more closely related to the *Echinostomum* group than to the tail-less cercaria group. So far as we know at present the *Gymnophallus* group is the only one in which the cercaria throughout has no tail, and this is a very well-marked group; the fork-tailed cercariae (*e.g. C. dichotoma*) however seem to be closely related. It seems to me therefore that the tail is not of so great importance primarily in classifying cercariae as is the question whether it develops in sporocysts or rediae and I have therefore made two primary groups:

I. Developing in sporocysts. *Gasterostomum* group. *Gymnophallus* group. *Cercaria dichotoma.* *Spelotrema* group. Group of stumpy-tailed cercariae. *Lepodora.*

II. Developing in rediae. *Cercaria neptuneae.* *Acanthopsolus.* *Echinostomum* group. *Monostomum* group.

From this classification I have omitted all fresh water forms that are not also marine and have restricted myself to those already known in Britain.

We divide the digenetic Trematodes into *Gasterostomata* and *Prostomata* according to whether the oral sucker is median or, as is usually the case, at the anterior end. This division is as clearly marked in the larval forms as in the adults. As well as having the sac-like intestine opening in the centre of the body the Gasterostomatous cercaria, which is known as *Bucephalus,* is provided with a peculiar and very characteristic tail having two long lateral filaments by aid of which it swims.

The *Prostomata* may then be sub-divided into groups some of which stand out much more conspicuously than others, but this is probably because they are more completely known; such groups are the *Spelotrema* group and the *Echinostomum* group, both of which are parasitic in birds. Others are difficult to place such as *Lepodora* and *Cercaria neptuneae*, but very little is known of these and further investigation will no doubt elucidate matters. When several species are known which undoubtedly belong to the same group we find them extraordinarily constant as to the kind of hosts they inhabit, thus all the members of the *Echinostomum* group so far as is at present known develop in rediae in the digestive glands of Gastropods, have a free-swimming stage and encyst in another molluscan host, in two cases known in the foot. Members of the *Spelotrema* group develop in sporocysts in Mollusca (Gastropods or Pelecypods) and encyst in Crustacea, except where the life-history is abbreviated and encystment takes place within the sporocyst in the first host. The members of the *Gymnophallus* group all seem to live for a time free between the mantle and shell of a Mollusk, usually a Pelecypod.

It seems to me that we know too little of these cercariae to attempt at present to classify them further in relation to their adult forms. I therefore have divided up the *Prostomata* into certain groups each of which is characterised in some definite way, but it is not intended to show any relationships between these groups other than the general resemblance of their larval stages.

I. GASTEROSTOMATA.

Cercaria developed in sporocysts.

Sporocysts occur in Pelecypoda, long and winding, branched or unbranched.

Cercaria known as *Bucephalus*.

Cystogenous organ anterior, ventral sucker opening into sac-like intestine. Tail a broad case with two lateral, very contractile and long filaments for swimming.

Intermediate host—a fish.

Final host—a fish.

II. PROSTOMATA.

A. (Two suckers.)

1. *Developed in sporocysts.*

(*a*) *Gymnophallus* group. Cercaria tail-less, developed (in the only species in which the sporocysts are known) in more or less spherical sporocysts in Pelecypoda. Oval body covered with spines.

No prepharynx, conspicuous pharynx : short and broad intestinal caeca seldom reaching beyond ventral sucker. Ventral sucker always behind centre of body. Excretory vesicle large, forked, reaching far forward anteriorly.

Intermediate host usually a Pelecypod. Cercaria lives between mantle and shell, finally settling down in the tissues of its host which forms a cyst round it of epithelial cells, the cercaria itself not forming a cyst.

Final host a bird, usually of the Duck tribe.

(*b*) *Group of fork-tailed Cercariae.* These seem closely allied to *Gymnophallus* but further life-history not known. Cercaria very like *Gymnophallus* but with excretory vesicle continuing down tail and opening at end of each fork. Develops in sausage-shaped sporocysts in Pelecypoda.

(*c*) *Spelotrema* group. Cercaria developed in sporocysts, spherical or oblong, in Gastropods. May have a free-swimming stage and in that case (*e.g. Spelotrema excellens*) is very small, oval and transparent with a thin tail about as long as or slightly longer than the body, provided with a boring stylet anteriorly and large stylet glands. If free-swimming stage is not present the cercaria is like the adult, tongue-shaped, covered more or less with spines, with long prepharynx and oesophagus, short intestinal lobes, not reaching to end of body. Tail may be completely absent or present in young stages of those which encyst in the sporocysts.

If intermediate host is required it is a Crustacean in which the cercaria encysts.

Final host—a bird.

(*d*) *Group of stumpy-tailed Cercariae.* Cercaria developed in sausage-shaped sporocysts, often brightly coloured, in Gastropoda. Very small and transparent. Suckers almost of the same size. Oral sucker provided with a stylet and conspicuous stylet glands. Excretory vesicle very thick-walled. Tail very broad and stumpy.

Further life-history unknown.

(*e*) *Lepodora.* Only one species of cercaria known, oval, covered with spines. Intestinal lobes reaching nearly to posterior end of body. Tail present in young form, cast off before encystment which takes place within the sausage-shaped sporocysts in a Pelecypod.

Final host—a fish.

2. *Developed in rediae.*

(*a*) *Cercaria neptuneae.* Cercaria developed in redia in a Gastropod, very thick and large tail, two eye-spots present. Stylet glands (?) and cystogenous glands. Excretory vesicle very thick-walled.

Further life-history unknown.

(*b*) *Acanthopsolus.* Cercaria developed in redia in a Gastropod, two eye-spots, intestinal lobes reaching nearly to posterior end of body, thin tail developed in young forms which is cast off before it is full grown.

Encysted stage and intermediate host probably omitted.

Final host—a fish.

(*c*) *Echinostomum* group. Cercaria developed in redia in a Gastropod. Redia usually coloured yellow or orange, provided with a collar, birth-pore and ambulatory processes. Cercaria with well-marked head end provided with crown of spines. Intestinal lobes reaching nearly to posterior end of body. Excretory system with small posterior vesicle and two conspicuous lateral canals. Tail long and narrow. Free-swimming stage.

Intermediate host—a Mollusk or fish.

Final host—Mammal, bird or fish.

B. (With one sucker only.)

(*d*) *Monostomum* group. Cercaria developed in rediae in a Gastropod. Eye-spots, tail long. Free-swimming stage. Encysted stage perhaps free.

Final host—a bird or fish.

Description of Material.

Nearly all the cercariae referred to in the following notes have been seen by myself and drawings made from the living objects. A few have been copied from others when I could not get specimens, and for these I have to thank Dr William Nicoll and Mr Jas. Johnstone.

Most of the work has been carried on at the Dove Marine Laboratory, Cullercoats, through the kindness of Armstrong College, Newcastle-upon-Tyne. Part of it was done at the Marine Station, Millport, and the Marine Laboratory, Plymouth, and I have also done a good deal of it at Leeds University.

In the course of my work I have examined material at many different times of year, and find that in nearly every case the sporocysts, rediae and cercariae are formed most abundantly in the spring, summer and early autumn,—scarcely at all in the winter. The encysted stages, however, are to be found through the winter, and it seems that these are real resting stages in which the larva has ceased to feed and is gradually forming its reproductive organs and getting ready for its adult life.

Effect of Parasite on Host.

The presence of sporocysts and rediae certainly injures the Molluscan host. The outer appearance of the digestive gland is usually enough to show if it is inhabited by these parasites. It looks unhealthy and is a grey, sickly yellow or a peculiar unnatural orange. It is generally completely riddled with the sporocysts or rediae which feed upon its substance. If the gonad is infected it is quite destroyed.

On the other hand encysted cercariae seem to do little or no harm to their hosts even when present in great numbers; they may even be of economic importance, as in the formation of pearls.

Many larval Trematodes infest our edible shell-fish. For instance *Bucephalus haimeanus* entirely spoils the cockles which it occupies. Many sporocysts and rediae occupy the digestive gland of periwinkles and *Buccinum* rendering it not a digestive gland at all but simply an organ full of parasites. The edible crab *Cancer pagurus* is also very often crowded with the cysts of *Spelotrema excellens* although this does not appear to affect the tissues in any way.

The following is a list of Trematode larvae occupying some of our most important edible shell-fish:

(1) In *Littorina littorea* (the periwinkle)
    Cercaria linearis (kidney and mantle cavity)
    Echinostomum secundum (digestive gland)
    Cercaria lophocerca (digestive gland)
(2) In *Buccinum undatum* (the "buckie")
    Cercaria buccini (digestive gland)
    C. neptuneae (digestive gland)
    Acanthopsolus lageniformis (digestive gland)
(3) In *Cardium edule* (the common cockle)
    Bucephalus haimeanus (throughout body)
    Gymnophallus margaritae (under umbo)
    Lepodora rachiaea (?) (visceral mass)
    Echinostomum secundum (foot)

(4) In *Mytilus edulis* (the common mussel)
Echinostomum secundum (foot)
Gymnophallus margaritae (between mantle and foot)
(5) In *Cancer pagurus* (the edible crab)
Spelotrema excellens (all over body).

Systematic Section.

I. GASTEROSTOMATA.

Genus *Gasterostomum*

Gasterostomum gracilescens (Rud.) (Lebour, 1908 B, p. 8).

Larval form *Bucephalus haimeanus* Lacaze-Duthiers.

(Pl. XXV, figs. 1–4.)

This is the well-known oyster parasite. In Britain however it has only been observed in the cockle *Cardium edule* L., but always in the vicinity of oyster beds. It occurs in two per cent. of the cockles from Fenham Flats, Northumberland, and more commonly in the same Mollusk from Emsworth, Hampshire (in about 10 per cent. of those which live near the oysters). For the specimens I am indebted to Miss Florence Jewell of Emsworth, who kindly sent me them on several occasions. Johnstone (1904, p. 106) has recorded a specimen of *Cardium edule* from the Lancashire coast infested with the parasite.

The cercaria occurs in very long sporocysts which are irregularly branched at intervals and so tangled together that it is almost impossible to separate them (fig. 1). They are packed closely together all over the body except the foot, completely destroying the gonad. Inside the sporocysts are the *Bucephalus* cercariae in all stages. The full-grown cercaria (fig. 2) measures 0·26 mm. in length without the tail which is extremely contractile and may be many times longer than the body. The tail is a very characteristic organ consisting of a triangular median portion at the hind end of the body bearing on each side a lateral process which is thin and elastic, alternately contracting and expanding as the cercaria swims. The body is covered, except at the tail, with minute spines arranged in transverse rows which give it a striated appearance. The mouth is in the centre of the ventral sucker which leads to a simple sac-like intestine. At the anterior end of the body is a sucker-like cystogenous organ. Posteriorly is a clear pear-shaped excretory vesicle.

The encysted stage of what is almost certainly the same worm occurs in the nerve cord and nerves of various Gadidae, most commonly the haddock *Gadus aeglefinus* L. Williamson (1911, p. 60) has recently recorded these cysts from the skin as well as the nerves of Gadidae. It was Dr David Hilt Tennant (1906, p. 635) who, working at the life-history of the American *Bucephalus haimeanus*, discovered its identity with some species of *Gasterostomum*, but I doubt if it be the same species as the British form although very similar. He found the encysted stage in the silverside *Menidia menidia* and the adult in the gar *Tylosaurus marinus*. The cysts in the nerves of the haddock are the "Bodies of Munro" of Maddox (1867, p. 87). They occur particularly in the region of the auditory and spinal nerves. They are also common in the cod *Gadus morrhua* L., and the whiting *G. merlangus* L., Johnstone (1904, p. 101) records it from the Lancashire coast in *Phycis blennioides* and also from the cod and haddock.

The cysts (fig. 3) are oval and thin-walled appearing as little bead-like swellings along the nerves. Length of cyst 0·60 mm. The cercaria coiled up inside is quite colourless with a very conspicuous opaque excretory vesicle. When pressed out of the cyst it measures about 2·5 mm. in length and is covered with minute spines (fig. 4). At about the first quarter of the body is the ventral sucker leading to a sac-like intestine. At the anterior end is a sucker-like organ. The excretory vesicle is long and narrow not reaching quite so far as the ventral sucker. A pair of testes is situated obliquely one behind the other in the posterior third of the body, the two ducts from these uniting in a common vas deferens which is continuous with the posteriorly situated vesicula seminalis and cirrus sac, the genital pore opening at the hind end of the body. The ovary is situated between the testes and the ventral sucker; the uterus and shell gland complex are present though not fully formed and no eggs are as yet developed. This encysted form is certainly identical with *Gasterostomum gracilescens* which lives in great numbers in the stomach and pyloric caeca of the angler *Lophius piscatorius*; all stages can be traced in it from forms exactly corresponding with the cercaria pressed out of the cyst from the haddock to the adult form full of eggs. The angler feeds on fish of all kinds, notably Gadoid, and so easily gets the worm into its alimentary canal. Nicoll (1909, p. 24) found immature specimens of this worm in the stomach of a cod. As he says, it probably got there by its swallowing some other Gadoid, and almost certainly it would not develop any further in the cod.

Gasterostomum sp.

Bucephalus syndosmyae sp. inq.

(Pl. XXV, figs. 5, 6.)

This cercaria occurred once out of five specimens of *Syndosmya alba* from Millport, Scotland, in July. Pelseneer (1906, p. 176) records *Bucephalus haimeanus* from this Mollusk, but this is certainly distinct.

The sporocysts riddled the whole animal but especially the digestive gland and region of the gonad. They are very long, thread-like and unbranched and contain cercariae in all stages (fig. 5). The full-grown cercaria which was bursting from the sporocyst is very like *B. haimeanus* (fig. 6). The ventral sucker (0·01 mm. across) which leads to the sac-like intestine is behind the centre of the body instead of in front. The body is covered with small spines. Anteriorly is a sucker-like cystogenous organ 0·03 mm. long by 0·02 mm. broad, or circular according to the state of contraction. Posteriorly is the tail with long processes. The centre of the tail is partially divided posteriorly by an indentation.

Further stages of the life-history are not known.

Besides these cercariae are to be noted encysted Gasterostomatous Trematodes found by Johnstone (1904, p. 103) in the muscles of small plaice. These were sexually mature and the uterus was full of eggs. This worm seems more nearly related to the genus *Prosorhynchus* than to *Gasterostomum*.

II. PROSTOMATA.

1. *Cercariae developed in sporocysts.*

(*a*) *Gymnophallus* group.

Genus *Gymnophallus* Odhner.

Cercaria margaritae sp. inq. (= the Pearl Trematode of Jameson (1902)).

(Pl. XXV, figs. 7, 8.)

This is referred by Jameson to *Leucithodendrium somateriae* (Levinsen) but Odhner (1905, p. 312) thinks it resembles *Gymnophallus bursicola* more closely, and Nicoll (IX. 1907, p. 265) is of the opinion that it may be *G. dapsilis*. It is extremely difficult without infection experiments to say which it is when so many of these closely similar species are common in the same host.

The young cercaria occurs in sporocysts in *Cardium edule* and *Tapes decussata.* The sporocysts are simple, more or less spherical sacs, quite colourless, some large and some small, Jameson (1902, p. 153) found them in *Tapes* in the muscular or connective tissue of the mantle margin, particularly dorsally, close to the siphonal muscles. He thinks that they increase by budding and grow as the Mollusk grows. In between the sporocysts are often to be found chalky spots, the development of which Jameson regards as due to the compression of the tissues, owing to the growth of the sporocyst and this interferes with the secretion of fresh shell layers; hence these white chalky masses.

Cardium edule was also found by Jameson to be a host for the sporocysts and these also occurred in the mantle margin. Afterwards Nicoll (I. 1906, p. 149) at St Andrews and myself (1907 A, p. 18) in Northumberland found these sporocysts very frequently in *Cardium edule* but not usually in the mantle margin. The usual habitat was just under the umbo where they occurred as a jelly-like mass frequently with the chalky spots mixed with them. The cockles of Fenham Flats and Budle Bay, Northumberland, were very commonly infected by this parasite. I also found it in the cockles at Millport.

The sporocysts (fig. 7) contain the cercariae (fig. 8) which measure when fully formed 0·15–0·3 mm. in length, the body covered with spines, a pair of brown eye-spots at the anterior end, one on each side of the oral sucker. The ratio of the oral to the ventral sucker is about 2 : 3. The lobes of the intestine do not reach so far as the ventral sucker and have no food inside. The testes and traces of the other reproductive organs can be seen in dead specimens only. The excretory sac is large with its lateral forks reaching as far as the pharynx.

The cercaria presumably leaves its first host and by aid of currents or by crawling on the ground by aid of its suckers, reaches its intermediate host, *Mytilus edulis.* Here it settles down between the mantle and shell, feeds and grows and finally makes its way into the tissues of the mantle, curls itself up and rests. It does not itself secrete any cyst but the mantle cells grow round it and form an enclosing sac of epithelium. Jameson believes that the parasite stimulates the mantle parenchyma to form these epithelial-like cells which are capable of secreting a pearly-layer round the parasite. The cercaria may make its escape from the sac before the pearly-layer is formed otherwise it dies forming a nucleus for the pearl.

The final host is almost certainly one of the ducks. Jameson believes it to be the scoter *Oedemia nigra* L. which eats mussels voraciously. Nicoll's *G. dapsilis* was also from the scoter *O. nigra* and the velvet scoter *O. fusca*.

Cercaria scrivenensis sp. inq.

(Pl. XXV, fig. 9.)

This was found on one occasion in *Tapes pullastra* from Loch Scriven, the Clyde, in August. Two examples occurred in one individual between the mantle and shell. It appears to be different from the other *Gymnophallus* cercariae so far known. Length 0·4 mm., breadth 0·24 mm. Body covered with spines. Oral sucker 0·09 mm. across, ventral sucker 0·03 mm. across. Intestinal caeca reaching a good way beyond the ventral sucker. Pharynx 0·03 mm. long. Excretory system conspicuous, forks reaching to pharynx.

Further life-history unknown.

Cercaria glandosa mihi (1908 A, p. 4).

(Pl. XXV, fig. 10.)

This occurs in *Paludestrina stagnalis* from Fenham Flats and is the only *Gymnophallus* cercaria known that inhabits a Gastropod. It is always free between mantle and shell. Only one to six specimens occur in the same individual. It is not certain whether this is the first or the intermediate host as no sporocysts were found. From the scarcity of specimens it seems more likely that it is the intermediate host.

The cercaria measures 0·20 mm. in length, very contractile, head region contains numerous gland cells filling up the space between body wall and oral sucker and opening by ducts round the dorsal margin of the oral sucker. It is possible that these are cystogenous glands and that in this form there is an encysted stage. Oral sucker 0·05 mm. across, ventral sucker 0·033 mm. with inner margin crenulated. Small pharynx leading to short oesophagus, intestinal caeca extending to beyond the centre of the ventral sucker, usually black with contained food. Excretory vesicle large, forks not reaching as far as pharynx. Testes developed on each side of excretory vesicle.

Further life-history unknown. Possibly this and not *C. margaritae* is the cercaria of *G. somateriae* which inhabits the intestine of the Eider Duck *Somateria mollissima*.

Cercaria macomae mihi (1908 A, p. 6).

(Pl. XXV, fig. 11.)

In *Macoma balthica* from Fenham Flats, occurring between mantle and shell, not more than three in any one specimen, three out of 20 examined contained them. The cercaria measures 0·70 mm. long. Oral sucker 0·22–0·26 mm. Ventral sucker 0·06–0·07 mm. with a crenulated inner margin. Small pharynx, short oesophagus, intestinal caeca reaching not quite to the level of the centre of the ventral sucker. Excretory vesicle horse-shoe shaped. Testes present, ovary in front of right testis, oval and slightly smaller than testis. Vitellaria lie below and to right side of ventral sucker.

Further life-history unknown.

Cercaria strigata mihi (1908 A, p. 7).

(Pl. XXV, fig. 12.)

In *Tellina tenuis* and *Donax vittatus* from Alnmouth, Northumberland, between mantle and shell in every specimen of *Tellina* examined (about 20) and in one *Donax* out of a dozen. It also occurs in 3 per cent. of *Tellina tenuis* at Millport. The body spines are very conspicuous and stand out in ridges giving the worm a striated appearance. Length 0·30–0·40 mm., oral sucker 0·09 mm., ventral sucker 0·052 mm. Small pharynx, 0·03 mm. long, short oesophagus, intestinal caeca broad and almost circular, reaching a very little way beyond the anterior margin of the ventral sucker. Excretory vesicle lyre-shaped. Testes sometimes to be seen one on each side of excretory vesicle. Hind part of body peculiar in being slightly drawn out at the end so as to give it the appearance of having a short tail. Giard (1907, p. 416) regarded this cercaria as very probably the young of *G. somateriae*, I think however, as I have suggested in a former paper (1908 A, p. 9), that it is more likely to be the young form of some *Gymnophallus* inhabiting a bird other than a duck, perhaps a gull.

(*b*) *Fork-tailed Cercariae.*

Cercaria dichotoma Müller.

(Lebour 1908 A, p. 13.)

(Pl. XXVI, figs. 1, 2.)

This cercaria seems to be closely allied in structure to *Gymnophallus* although the presence of the forked tail seems to warrant its being placed in a separate group[1]. It occurs in long irregular sporocysts in the little bivalve *Scrobicularia tenuis* from Fenham Flats in 1 out of 50. The sporocyst measures about 1 mm. in length, very transparent and full of cercariae in all stages. The cercaria measures 0·20 mm. in length with the tail which is slightly shorter than the body and forked at its free end. Oral sucker 0·033 mm. across leading directly to pharynx, 0·016 mm. long. Rather long oesophagus, intestinal caeca reaching a little way beyond the anterior margin of the ventral sucker. Ventral sucker lying well behind the centre of body, 0·029 mm. across. Excretory vesicle lyre-shaped, occupying most of the posterior part of body and continued right along tail where it opens at the end of each fork.

Johnstone (1904, p. 107) records it from *Cardium edule* from the Lancashire coast.

The further life-history is not known. I should say the adult must be closely related to *Gymnophallus*. The form of the intestine, excretory vesicle and position and relations of the suckers all indicate a near relative of this genus. The final host is almost certainly a bird.

(*c*) *The Spelotrema group.*

The adults of this group all live in birds and are very much alike. The intermediate host if present is a Crustacean in which the cercaria encysts. The cercaria in the cyst can always be recognised as belonging to this group as it is very like the adult except that the reproductive organs are not fully developed. The typical cercaria, like the adult, is tongue-shaped, covered more or less with spines, more pointed anteriorly than posteriorly, very much flattened dorso-ventrally, suckers nearly equal, prepharynx usually long, pharynx long, oesophagus branching into short intestinal caeca which rarely reach beyond the ventral sucker. It is generally difficult to make out much more of these cercariae and therefore it is hardly possible to ascribe them to different genera. The genera *Spelotrema*, *Maritrema*, *Levinsenia*, *Levinsiella* are very much alike and are only differentiated by their reproductive and excretory organs. It is sometimes easy to see the genital opening and papilla in the *Spelotrema* cercaria, and in one species *S. excellens* Nicoll I have been able to follow out most of the life-history.

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[1] Odhner (1911, p. 105) describes a fork-tailed cercaria which he believes to be a species of *Haplocladus*.

Some forms of this group encyst in their sporocysts in Gastropod hosts and these I think may possibly belong to the genus *Maritrema*.

There are two distinct kinds of cercariae in the *Spelotrema* group. One in which the cercaria has a free-swimming stage and is provided with a stylet and stylet glands. This is at first quite unlike the encysted form and gradually assumes the adult characteristics within the cyst. Such cercariae occur in *S. excellens* and *S. balani*. The other kind is like the encysted form even within the sporocysts, apparently possessing no tail at any period of its existence. This is the case with *Cercaria littorinae-rudis*. In such forms which encyst in the sporocyst the cercaria is at first tailed but gradually assumes the ordinary *Spelotrema* form, loses its tail and encysts.

Genus *Spelotrema* Jägerskiöld.

Spelotrema excellens Nicoll (x. 1907, p. 248).

(Pl. XXVI, figs. 3–10.)

The encysted cercaria which for excellent reasons Nicoll regards as *S. excellens* occurs in the common green crab *Carcinus maenas* in almost every organ and seems to be universally distributed in Britain. The first to describe this cercaria from the crab was McIntosh (1865, p. 201) who found it abundantly at St Andrews from the crabs in the rock pools. It was afterwards described by Nicoll and Small (1909, p. 238) from Millport, and I have found it commonly at Plymouth and on the Northumberland coast.

The first host of this Trematode has not as yet been described but I believe the very active and extremely small cercaria which is common in many places and which I have named *Cercaria ubiquita* (1907, p. 444) to be a stage in the life-history. It never occurred to me to identify this little creature with the encysted *Spelotrema* from the crab until after pondering over the question of its life-history at Millport and examining many Mollusca and crabs I was able to follow up the various stages of *Cercaria ubiquita* and found that it entered the crab, and there underwent changes until it finally turned into a *Spelotrema*-like cercaria.

Cercaria ubiquita is common in *Paludestrina stagnalis* at Fenham Flats and Loch Ryan on the West Coast of Scotland. Occasionally also I have found it in *Littorina obtusata* and *Littorina rudis.* At Millport *Littorina obtusata* from the rock pools where the crabs live is its usual host. It occupies the digestive gland in oval sporocysts and the whole of the organ has an unhealthy yellowish colour and can generally be quite easily told from an ordinary healthy digestive gland.

The sporocysts (fig. 3) are oval and of various sizes, the usual size being about 0·6 mm. long. They are pinkish yellow when together but almost colourless when looked at singly. Cercariae in all stages are contained within which easily burst out of the thin-walled sporocyst.

The cercaria (fig. 4) measures 0·12 mm. in length without its tail which is about the same length as the body and very thin and active. It is extremely transparent. The oral sucker measures about 0·030 mm. across but often appears oval when extended. It is armed with a long pointed stylet and by its sides open the four ducts (two each side) of the stylet glands which run up the body springing from two masses of large cells which occupy the greater part of the body. No ventral sucker is to be seen. It apparently develops afterwards as does also the alimentary canal. The excretory vesicle is bilobed. The tail very easily detaches itself and moves about separately. The cercaria is fond of doubling itself up with its fore and hind ends doubled in so that it looks like a neatly folded parcel. It apparently leaves its first host and swims freely about in the water, then bores its way by means of its stylet and glands into the tissues of the crab. The edible crab *Cancer pagurus* is also an intermediate host for this worm. I have found cercariae exactly corresponding with *C. ubiquita*, but without the glands, inside the tissues of *Carcinus maenas.* I have also found it curled up and surrounded by a very thin cyst, 0·08 mm. across (fig. 5). It has now lost its tail but the stylet remains for some time longer. It occupies almost every tissue of the crab, liver, muscles, gonad and outside the blood vessels. Having settled down it grows considerably and the cyst with it, but the latter however is still very thin-walled. The stylet is lost when the cyst measures about 0·30 mm. across. The ventral sucker and alimentary canal appear and the body spines begin to form. The worm stops growing when the cyst is about 0·35 mm. across and then the cyst wall becomes very thick, 0·02 mm. thick and the real resting stage begins. The cercaria is now of the ordinary *Spelotrema* form. The usual size of the thick-walled cyst is 0·4–0·48 mm. across. It is very difficult to get the enclosed worm out of the cyst without injuring it as the cysts are extraordinarily difficult to break through.

The cercaria when pressed out of the cyst measures about 0·8 mm. in length. Its body is covered with spines except at the posterior end. Oral sucker 0·066 mm. across, slightly larger than the ventral sucker. Prepharynx long, pharynx conspicuous, oesophagus long branching into two caeca which reach to about the level of the centre of the ventral sucker. The genital papilla is plainly to be seen on the left side of the ventral sucker and often the vesicula seminalis is apparent. The excretory vesicle is large and specially conspicuous in the hind part of the encysted cercaria, two lateral ducts are plainly to be seen opening into it. Testes one on each side behind ventral sucker. From what has been said there can be little doubt that *Cercaria ubiquita* is the young form of *S. excellens* the first host thus being *Paludestrina stagnalis, Littorina obtusata* and *L. rudis.* The intermediate host *Carcinus maenas* and *Cancer pagurus* and the final host probably the herring gull *Larus argentatus.*

The sporocysts and cercariae are not to be found in the winter and the crabs in the winter only contain the thick-walled cysts. The small thin-walled cysts are only to be found in the spring, summer and early autumn. This points to the summer and autumn as the time for the migration of the cercariae from the first to the intermediate host. The passing from *Carcinus* to its final host may almost certainly take place at any time of year.

The cercariae *C. cellulosa* and *C. pusilla* of Looss (1896, p. 227) probably belong to the *Spelotrema* group as they are very like *Cercaria ubiquita.* In that case the final host would be a bird and not an insectivore as the author suggests.

There are at least two other forms belonging to the *Spelotrema* group which encyst in the crab but which I have not been able to investigate in any detail. These are *Cercaria carcini* mihi and another small one which I have named *Cercaria minor.* The encysted cercaria with thick cyst mentioned by myself from *Carcinus* (1908 A, p. 11) is probably an unusually small example of *S. excellens.*

Cercaria carcini mihi (1908 A, p. 10).

(Pl. XXVI, figs. 13, 14.)

This was found encysted in *Carcinus maenas* from Fenham Flats. Only a few specimens were examined but in no case were the cercariae frequent. The cyst is oval and thin-walled, 0·40 × 0·35 mm. The cercaria when pressed out of the cyst measures 0·54 mm. in length, suckers almost equal, intestinal lobes reaching to the level of the anterior margin of the ventral sucker. Body covered with spines except at the posterior end. This is very like *S. excellens* but I think from the character of the cyst that it is a different species.

Cercaria minor sp. inq.

(Pl. XXVII, figs. 1, 2.)

Occurs in smaller cysts than *S. excellens* but with them in *Carcinus maenas*. The cyst wall is much thinner. Breadth of cyst 0·26 mm. The cercaria contained within it is well developed and certainly distinct from *S. excellens*. Length 0·52 mm. Body covered with spines except at the posterior end. Oral sucker 0·46 × 0·40 mm., broader transversely. Ventral sucker 0·40 mm. Prepharynx and oesophagus long. Intestinal caeca reaching to just beyond the posterior border of the ventral sucker. Genital pore to left of ventral sucker. Testes and vitellaria behind.

The further life-history is not known.

Cercaria corophii mihi (1908 A, p. 9).

(Pl. XXV, figs. 18, 19.)

This cercaria occurs frequently encysted near the surface of the body and legs of the amphipods *Corophium grossipes* and *Gammarus duebeni* especially in the former, just beneath the cuticle. All those found were from Fenham Flats. The cyst is slightly oval 0·16 × 0·14 mm., with thick transparent walls. When pressed out of the cyst the cercaria measures 0·34 mm. in length, broad at the base with head end tapering. Body covered with spines except at the posterior end. Oral sucker 0·03 mm. across, narrow prepharynx 0·04 mm. long, pharynx 0·016 mm., long oesophagus branching into caeca reaching just beyond the posterior border of the ventral sucker. Ventral sucker 0·023 mm. across.

Nothing further of the life-history is known.

Cercaria balani mihi (1908 A, p. 10).

(Pl. XXVI, figs. 15, 18.)

Occurs encysted in the common barnacle *Balanus balanoides* from Budle Bay and Fenham Flats. The cyst is thin-walled and spherical (fig. 16), 0·04 mm. across. The cercaria is pale yellow and when pressed out of the cyst measures about 1 mm. in length. Very long prepharynx, longer than the oesophagus. Intestinal caeca reaching to just beyond the posterior border of the ventral sucker. Body covered with spines even at the posterior end.

It is interesting to note that small cysts with very thin walls are found with these large forms in *Balanus* and inside are curled up cercariae with stylets very like *Cercaria ubiquita*. This seems to show that the same kind of cercaria enters the *Balanus* and grows into the *Spelotrema* form.

Some time ago I suggested (1908 A, p. 10) that this was the encysted *Spelotrema excellens* but now think that the form so commonly found in the crab is much more likely to be this species.

The further life-history is not known.

Cercaria littorinae-rudis sp. inq.

(Lebour, 1906, p. 6.)

(Pl. XXVII, figs. 3–7.)

This cercaria was found only once occupying the digestive gland of a specimen of *Littorina rudis* from Holy Island, Northumberland, in the beginning of May. It is a specially interesting form as it shows a Trematode most certainly belonging to the *Spelotrema* group but yet with such a different form of cercaria, for no tail was observed and it probably has none. In form of the sporocysts and habit of the cercaria it is much more like *Gymnophallus*. Pelseneer (1906, p. 171) found in *Natica alderi* from Boulogne cercariae very much like these in similar sporocysts but there were much fewer cercariae in each.

The sporocysts are pale yellow and spherical about 1 mm. across and are full of cercariae more or less doubled up. This doubling up or folding is interesting as they closely resemble *Cercaria ubiquita* in this way. The cercaria (fig. 5) when extended measures 0·25 mm. in length and the body is covered with spines except at the posterior end. Unfortunately I have not got more detailed measurements of this species. The prepharynx is much shorter than the oesophagus, intestinal caeca reaching to the posterior end of the ventral sucker. Genital opening on the left of the ventral sucker. Nicoll (IX. 1907, p. 255) considers this as very likely to be the larval form of *Spelotrema claviforme* (Brandes) from the dunlin *Tringa alpina* and the ringed plover *Aegialites hiaticula*.

The intermediate host is not known.

Cercaria oocysta mihi (1907 C, p. 445).

(Pl. XXVII, figs. 8, 9.)

Occurs encysted in sausage-shaped sporocysts in *Paludestrina stagnalis* from Fenham Flats and Loch Ryan. It occupies the digestive gland in 50 per cent. of those from Fenham Flats and two per cent. from Loch Ryan. The sporocysts are colourless and very thin-walled enclosing cercariae in all stages. The younger cercariae are tailed but the tail is soon lost and the worm settles down to encyst.

The cyst is thin-walled and a long oval 0·12 × 0·07 mm. The cast-off tails are to be seen moving about within the sporocyst. When all the cercariae are encysted the wall of the sporocyst dwindles and disappears.

The cercaria measures 0·24 mm. in length when pressed out of the cyst and is about $3\frac{1}{2}$ times as long as it is broad. The body is covered all over with spines. Oral sucker 0·026 mm. across, pharynx almost mid-way between the oral sucker and the bifurcation of the intestine but slightly further forward. Intestinal caeca reaching to about the level of the centre of the ventral sucker. Ventral sucker 0·019 mm. across. Lateral excretory canals can be made out faintly in the live worm. A cirrus sac opens on the left of the ventral sucker. Vitellaria and testes developed.

This cercaria was found in August at Loch Ryan and in the winter from Fenham Flats.

Further life-history not known, perhaps it is a species of *Maritrema*.

Cercaria pirum mihi (1907 C, p. 446).

(Pl. XXVII, figs. 10, 11.)

Occurs in the digestive gland of *Paludestrina stagnalis* in about four per cent. from Fenham Flats in July, October and December. The cercaria occurred in cysts all over the digestive gland and in those specimens examined in July the remains of the walls of the sporocysts could be seen which were just breaking away. In the autumn and winter all traces of the sporocysts had gone and the cysts were quite free.

The cyst is spherical, 0·08 mm. across, quite transparent and colourless. The cercaria when pressed out of the cyst is 0·2 mm. long and very broad at the base. It is covered all over with small spines, more conspicuous near the head which appears very rough. Oral sucker 0·026 mm. across, ventral sucker about the same size occurring about three parts of the way down the body. Prepharynx very much shorter, long oesophagus, intestinal caeca reaching to about the level of the posterior border of the ventral sucker. The two lateral excretory canals unite behind the pharynx.

Nothing further is known of the life-history. It is very likely a species of *Maritrema* and perhaps the habit of encysting in the sporocyst may distinguish this genus from *Spelotrema.*

(*d*) *Stumpy-tailed Cercariae.*

Three different species belonging to this very well-marked group occur in Britain, one of which I have already recorded (1906, p. 5) *e.g. Cercaria linearis* Lespés, one which I now record as new to Britain *C. brachyura* (Lespés) and one new species. All are characterised by their very short stumpy tails which are incapable of independent movement and probably serve to attach the animal (acting as a sucker) whilst it is looking out for its intermediate host. The oral sucker is armed with a stylet and the stylet glands are conspicuous. The suckers are almost equal, the ventral sucker situated near the posterior end. The excretory vesicle is extremely thick-walled; the large lining cells making it appear almost solid.

Cercaria linearis Lespés (1857, p. 113).

(Pl. XXVII, figs. 12, 13.)

This cercaria occurs in *Littorina littorea* from Budle Bay and also from Millport. It is rare however, only being found in one specimen out of 190 examined from Budle and in one out of about the same number from Millport, both in July.

The sausage-shaped sporocysts are very inert and occupy the kidney and mantle cavity spreading also into most of the other organs. They measure 2·6 mm. in length and are a brilliant orange, the colour being due to granular masses scattered throughout the sporocyst. The contained cercariae are colourless measuring 0·18 mm. in length including the tail, tail 0·015 mm. nearly square. Oral sucker 0·035 mm. armed with a stylet anteriorly cleft. By the side of the stylet open the four ducts of the stylet glands. The glands are conspicuous in front of the ventral sucker which measures 0·04 mm. across. The excretory vesicle is lined by very large conspicuous cells and opens posteriorly. The alimentary canal is not developed.

Further life-history not known.

Cercaria brachyura Lespés (1857, p. 113).

(Pl. XXVII, figs. 14–16.)

Occurs in the digestive gland of *Gibbula cinerea* from Loch Ryan and Millport. In two per cent. from Millport and only found once at Loch Ryan.

The sporocysts like the last species are a brilliant orange colour but they are very active and constantly change their shape. The young forms are redia-like although without pharynx and intestine, often showing processes like the ambulatory processes of a redia. This fact was noticed by Pelseneer (1906, p. 168) in what he takes to be this species[1]. The older redia measures 1·2 mm. in length. The cercaria without its tail measures 0·24 mm. in length. The oral and ventral suckers are nearly the same size, the oral slightly larger than the ventral, oral sucker 0·05 mm. across when circular but often appears longer than it is broad. I could not see the stylet mentioned by Lespés and Pelseneer does not mention it. The stylet glands however are very conspicuous in front of the ventral sucker, opening by four ducts near the oral sucker. A pharynx is sometimes to be seen preceded by a short prepharynx, but I could not see any more of the alimentary canal. The excretory vesicle is lined by very thick cells. The body in some of the specimens was covered with minute spines. The tail usually looks broader than long and is slightly cleft posteriorly.

I think it is very likely to be a different species from Pelseneer's as his ventral sucker is larger than the oral and there are other small differences. It may be distinct from Lespés' species also, but they are all very much alike.

The further life-history is unknown. Pelseneer observed some of his specimens encysted in *Gibbula cinerea.* The tails had disappeared in three out of four specimens. In the fourth it had just come off.

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[1] Pelseneer refers to it as *Cercaria pachycerca* Lespés.

Cercaria buccini sp. inq.

(Pl. XXVII, figs. 17, 18.)

This is the largest of the three cercariae belonging to this group. It occurs in *Buccinum undatum* brought in by the fishing boats, four out of 80 examined. It is contained in long and almost colourless sporocysts which occupy the digestive gland in enormous numbers. The gonad is not destroyed by this Trematode as is very usually the case with others.

The sporocysts measure 1·4 mm. to 3 mm. in length, pale yellow or colourless and sausage-shaped. They may be 20–30 cercariae in each sporocyst.

The cercaria measures 0·33 mm. including its tail, tail 0·06 mm., oral sucker 0·045 mm. across, armed with a stylet with four points anteriorly, the middle pair long and the outer short, bluntly rounded behind. The ventral sucker is situated well behind the centre of the body and is almost exactly the same size as the oral sucker. Between the two suckers are the stylet glands with six ducts, three on each side opening by the sides of the stylet anteriorly and dorsally. Behind the ventral sucker is a large excretory vesicle lined with very large cells.

Further life-history unknown.

(*e*) *Lepodora group.*

Genus *Lepodora.*

Lepodora rachiaea (Cobb.)? (Lebour, 1907 B, p. 102, and 1908 B, p. 20).

(Pl. XXVII, figs. 19, 20.)

The cercaria which is probably the larval stage of *Lepodora rachiaea* is very difficult to place in relation to any other larval forms. I have therefore left it at the bottom of the group developed in sporocysts.

It inhabits *Cardium edule* and is contained within sporocysts where it encysts. It was found twice by myself in cockles from Budle and Holy Island and once by Johnstone from Morecombe Bay completely riddling the visceral mass of the host.

The sporocysts are sausage-shaped and quite colourless. Sometimes they are active and constantly changing their shape, at other times they are quite inert. They measure about 0·60 mm. long and 0·15 mm. broad. Inside are cercariae in all stages, the older examples encysted in spherical thin-walled cysts 0·14 mm. across or smaller. The young cercariae are tailed (tail not quite so long as the body) and very active inside the sporocysts. The tails disappear some time before encystment.

The cercaria when pressed out of the cyst measures 0·19 mm. in length. The body is covered with spines arranged in transverse rows. Oral sucker 0·04 mm. across, short prepharynx, conspicuous pharynx, short oesophagus, lobes of intestine reaching nearly to the posterior end of the body. Small ventral sucker slightly behind the centre of the body. Large bilobed excretory vesicle.

The specimens were obtained in March, April and October. Tailed forms were only obtained in October. Those found in the spring contained the encysted cercariae in the sporocysts which possibly had been resting through the winter.

The final host of *Lepodora rachiaea* is the haddock which feeds much on cockles. This cercaria is very like *L. rachiaea* and is in all probability that species which passes directly into its final host by the haddock eating the cockle.

2. *Cercariae developed in Rediae.*

(*a*) *Cercaria neptuneae* sp. inq.

(Pl. XXVIII, figs. 1, 2.)

This is most difficult to place as in some respects it resembles the stumpy-tailed cercariae, in others it is like *Acanthopsolus*, and in the possession of a huge tail it is unlike either.

It occurs in the digestive gland of *Neptunea antiqua* brought in by the Cullercoats fishing boats, and also from *Buccinum undatum* from the same locality in July. In both the reproductive organs are destroyed. The whole spire is packed with the rediae which are colourless and measure about 2 mm. or more in length. The redia has a pharynx 0·04 mm. long, and a very short intestine, hardly longer than the pharynx. It contains the cercariae in various stages.

The cercaria measures 0·4 mm. in length without its tail, breadth in extended condition about 0·10 mm., but very variable. The body is covered with small spines. Tail exceedingly large and thick forming an effectual swimming organ, length about 0·26 mm., breadth when moderately extended about 0·049 mm. Oral sucker 0·033 mm. across when circular but it can be elongated considerably so as to be longer than broad. Prepharynx 0·06 mm. long, pharynx 0·03 mm. long, rest of alimentary canal not visible. Two large eye-spots just in front and to the sides of the pharynx. Ventral sucker at about the centre of the body, the same size or slightly smaller than the oral sucker. Large unicellular stylet glands at the sides of the ventral sucker opening by four long ducts near the oral sucker. A stylet was not seen but possibly there may be a very small one as these glands seem to indicate its presence. Another pair of glands just in front of the ventral sucker with long ducts are probably cystogenous glands. The very large excretory vesicle is oval and lined by large cells like those of the stumpy-tailed cercariae. This cercaria is very like the cercaria of *Allocreadium isoporum* Looss (1894, p. 54) which is developed in rediae in *Cyclas.* This has an enormous tail, eye-spots, prepharynx, pharynx and alimentary canal, conspicuous glands in the front part of its body. It is to be noted that the adult of this species has large unicellular glands ("head glands") opening near the oral sucker by long ducts. There is however an indication in Looss' figure of a stylet in the oral sucker of the cercaria.

It is just possible that *Cercaria neptuneae* may be the larval form of an *Allocreadium* or *Lebouria* Nicoll.

The further life-history is not known in any way.

(*b*) *Acanthopsolus* group.

Genus *Acanthopsolus* Odhner.

Acanthopsolus lageniformis mihi (1910, p. 29).

(Pl. XXVIII, figs. 3–6.)

Occurs in rediae in the digestive gland of *Buccinum undatum* in seven per cent. of those brought in by the Cullercoats fishing boats. Also it was found once in a specimen of *Buccinum undatum* from Holy Island.

The rediae are of various sizes, the older specimens being a pale yellowish. The smallest seen were about 0·30 mm. long and had no collar nor ambulatory appendages, hind end is pointed, pharynx and intestine conspicuous. These small rediae contained no cercariae. Larger specimens measuring from 0·50 mm. contained them, the intestine hardly growing at all, until in the large rediae of about 3 mm. it is so small that it can be easily overlooked. There may be 30 or more cercariae in each redia.

The young cercaria has a tail but it apparently loses it just before it is ready to emerge from the redia and in all probability there is no free-swimming stage. The tail is slightly longer than the body, thin and very active.

The full-grown cercaria measures about 0·50 mm. in length, greatest breadth slightly less than half the length. Form of the body oval or more or less flask-shaped, the fore end more pointed than the hind end. Body covered all over with spines although they dwindle posteriorly. Two eye-spots conspicuous at the front end. Suckers almost the same size (0·06 mm. across), the oral slightly the larger. Conspicuous prepharynx and pharynx, short oesophagus, intestinal caeca reaching nearly to the posterior end of the body. Just behind the oral sucker are some small gland cells. Ventral sucker almost in the centre of body. Excretory vesicle large and pear-shaped, sometimes slightly bilobed anteriorly. Testes one on each side midway between ventral sucker and posterior end of body. Ovary in front of right testis. Sometimes traces of male and female ducts.

The final host is the catfish *Anarrhichas lupus* and it is very probable that there is no intermediate host, the fish swallowing the *Buccinum undatum* with the contained rediae, the cercaria thus getting into the intestine without the aid of another host. I have never seen any trace of cysts in the catfish.

The cercariae were found in the spring and summer, never in the winter.

(c) *Echinostomum* group.

Genus *Echinostomum* Rud.

Head end provided with a crown of spines open posteriorly. Excretory system with much branched lateral canals. Intermediate host a Mollusk or in exceptional cases the cercaria may encyst in its first host. Final host usually a bird.

The species of this genus are often very much alike, frequently possessing the same number of head spines. Three different cercariae are known to me all of which have 29 head spines. They can be quite easily distinguished however, as the rediae in which they are developed are quite distinctly different. I have no doubt that they represent different species. Moreover they are contained in constantly different hosts. In support of this it is to be noted that Dietz (1910, p. 352) recognises four species of *Echinostomum* (all belonging to his genus *Himasthla*) which have 29 head spines. Odhner (1910, p. 137) however regards at least three of these as the same species.

Echinostomum leptosomum Creplin.

(Villot, 1898, p. 18. Lebour, 1907 C, p. 447.)

(Pl. XXVIII, figs. 7–10.)

Occurs in colourless or pale yellow rediae in the digestive gland of *Paludestrina stagnalis* from Fenham Flats and Loch Ryan. In about 26 per cent. from Fenham Flats and one per cent. from Loch Ryan. It occurs in spring, summer, and early autumn, most plentifully in marshy pools above high water mark, although also in pools left uncovered for several hours in the day.

The rediae are all sorts of shapes which are constantly changing; the smallest seen were 0·20 mm. long, the largest 1·4 mm. long. The young forms are pointed at the hind end and have two conspicuous ambulatory processes each side which tend to disappear in the larger rediae. The cuticle in all is very thick and much wrinkled anteriorly especially round the mouth. The pharynx is large but the intestine can hardly be made out. All stages of cercariae are contained within the rediae. The full-grown cercaria is tailed, without the tail 0·40–0·50 mm. long, tail 0·3 mm. long and very thin and active. It is extremely transparent, very agile and fond of bending itself backwards in the form of a U. Body covered with spines except at the posterior end. Head end with a crown of 29 head spines arranged in a row round three parts of the head two at each end being below the others but very little smaller (long spines 0·019–0·02 mm., short spines 0·016 mm.), oral sucker 0·030 mm. across, prepharynx rather long, pharynx 0·026 mm. long, oesophagus long and narrow with intestinal caeca reaching nearly to the posterior end of the body. Ventral sucker 0·046 mm. across. Excretory vesicle roundish, with two much branched lateral canals filled with clear granules. Ducts of glands opening near anterior end. Gland cells all over body. In one case an encysted cercaria was found among the rediae but this is an exception as there is an intermediate host. The cercaria leaves the first host and swims about, finally getting into *Scrobicularia tenuis*. This small bivalve is very common on Fenham Flats and the cercaria encysts in the foot as described by Villot. The cyst measures 0·12 mm. across with a thin transparent wall of two layers.

The final host is the turnstone *Strepsilas interpres* in which *Echinostomum leptosomum* is quite common. Villot found it in the dunlin *Tringa alpina* and I have also found it in this bird. Both feed on *Scrobicularia tenuis*.

Echinostomum secundum Nicoll (I. 1906, p. 151, III. 1906, p. 515),

(Lebour, 1905, p. 1, 1906, p. 3, and 1908 C, p. 354.)

(Pl. XXVIII, figs. 11–14.)

Occurs in the digestive gland of the common periwinkle *Littorina littorea* from Budle and Fenham Flats in about three per cent. in spring, summer, and early autumn, also in about five per cent. from Loch Ryan. The spire of the animal is always a brilliant orange and can easily be distinguished from healthy specimens which are not infected. The colour is due to the redia which is a bright pinkish orange when full grown. The very young rediae are colourless, the smallest seen was 0·40 mm. long, with a conspicuous pharynx and intestine, collar, and two ambulatory processes posteriorly. The anterior part between mouth and collar is grooved. The collar and grooves disappear and the ambulatory processes gradually dwindle in the full-grown redia, but there is always a trace of one of the processes, the redia having the shape of a stocking; it may be 2 or more mm. long, and is much more constant in shape than *E. leptosomum*.

The cercaria is colourless and transparent, length 0·70 mm. without tail which is not quite so long as the body. Body covered for the first two-thirds with small spines. Head armed with 29 spines arranged as in *E. leptosomum* but with the two short spines at each end, which are on a lower level, much shorter than the others. Oral sucker 0·065 mm. across, leading to a thin prepharynx, pharynx 0·05 × 0·03 mm., narrow oesophagus with intestinal caeca reaching nearly to the posterior end of body. Ventral sucker 0·095 mm. across. Excretory vesicle oval, two much branched lateral canals full of clear granules. Cystogenous ducts opening by oral sucker.

The cercaria leaves its first host and swims freely in the water until it reaches its intermediate host, which in this case is the common mussel *Mytilus edulis* in which it settles down and encysts in the foot. I have determined by experimental infection that this cercaria passes from the periwinkle to the mussel and encysts in the foot of the mussel (1909, p. 353). Cysts which are almost identical with these and probably the same species were also found in *Cardium edule*, *Mya arenaria* and *Tapes pullastra*. The cysts project from the foot in well-infected specimens as small papillae dotted all over it. Each cyst measures 0·21–0·25 mm. across, and, except for the size, is exactly like *E. leptosomum.* Almost every mussel at Budle contains these cysts and about 10 per cent. of the cockles. It is also common at Fenham Flats and Loch Ryan encysted in the mussels and cockles. The final host according to Nicoll (III. 1906, p. 515) is the oyster catcher *Haematopus ostralegus,* also the herring-gull *Larus argentatus* and the black-headed gull *L.ridibundus,* in which birds an *Echinostomum* occurs, which was traced by Nicoll from very young forms almost exactly corresponding to the above-mentioned cercaria to the adults, which he has described as *E. secundum.* I think from the evidence of the cercariae and rediae there is no doubt that these are distinct species. The third form which I believe to be also a distinct species is described below.

Echinostomum sp.

Cercaria littorinae obtusatae sp. inq.

(Pl. XXVIII, figs. 15–17.)

Occurs in the digestive gland of *Littorina obtusata* from Millport in about five per cent. It looks a pale yellowish-brown when infected but the redia is quite colourless and peculiar in having an extremely small pharynx and intestine even in the young forms. The smallest redia seen was 0·5 mm. long, of a squarish form with a conspicuous collar and more or less pronounced ambulatory processes. Pharynx very small with intestine not reaching half-way down the body. The older rediae measured 2–3 mm. in length, the pharynx so inconspicuous that it was difficult to see it at all and the intestine is actually smaller than in the youngest redia seen. The large rediae were full of cercariae in various stages.

The cercaria is extremely like *E. secundum,* the smaller head spines however are not so short as those in *E. secundum.* Length of body 0·78 mm., tail 0·4 mm. long. Body covered with spines for the first two-thirds of its length. 29 head spines. Oral sucker 0·06 mm. across, ventral sucker 0·08 mm. across. Excretory system with much branched lateral canals, the granules contained in them soon disappearing so that the clear canals are specially easy to see, and the part continued into the tail can clearly be made out bifurcating a little more than half-way down. In *E. leptosomum* and *E. secundum* it also is continued into the tail but cannot be so clearly seen.

The further life-history of this species is not known.

Cercaria purpurae sp. inq.

(Lebour, 1907 B, p. 102.)

(Pl. XXIX, figs. 1–3.)

This cercaria which I have before described from the dog-whelk *Purpura lapillus* undoubtedly belongs to the *Echinostomum* group although certainly to another genus. Having found it much more frequently than before I am able to describe it more in detail. It occurs in the digestive gland of *Purpura lapillus* from Budle Bay, Fenham Flats, Loch Ryan and Millport. It is the commonest at Millport in about 20 per cent. in spring, summer, and early autumn, but not in the winter. The infected gland is swollen and of a pale yellowish colour, packed full of rediae. The redia is pale yellow or colourless with a bright yellow intestine. The young forms are the most perfectly typical rediae that I know. They measure 0·32–0·60 mm. in length and are narrow and quite colourless. The pharynx is conspicuous and leads to a large intestine occupying about half the length of the body. Anteriorly and to the side is a thick-lipped birth-pore which is capable of protrusion or withdrawal. A collar is not present. Two very mobile ambulatory processes occur posteriorly one on each side of the pointed hind end, and these are also capable of being retracted completely or exserted for quite a long distance. These young rediae are to be found in the mantle cavity and also in the digestive gland and most probably migrate from one organ to the other. The sporocyst in all probability occurs in the mantle cavity and gives rise to rediae which migrate to the digestive gland where they feed and multiply enormously, finally giving rise to cercariae. The older redia loses its hind appendages but its pharynx and intestine are still conspicuous when it is full of cercariae. The full-grown redia may be 3·6 mm. long. The cercaria is very solid and thick but extremely active. Length of body 0·64 mm. without tail which is 0·42 mm. long. The tail is very thin with a sucker-like end. There is no indication of a continuation into it of the excretory system. Body entirely covered with broad scale-like spines except in the head region and the region of the ventral sucker. The inside of the body is occupied by large gland cells arranged in four groups, the ducts from which run together on each side expanding into six smaller ducts on each side which open by the oral sucker. The head is extremely complicated and beset with numerous spines and papillae. There is a considerable space, the neck, between the flat body spines and the head, and this head bear a row of fine spines arranged as far as I can make out as a crown undulating on the ventral surface, straight dorsally. The oral sucker has papillae arranged round its opening and there are also papillae round the oral sucker. Oral sucker 0·06 mm. across, short prepharynx, small but muscular pharynx. Oesophagus long, reaching to about the centre of the body where it bifurcates, intestinal caeca reaching nearly to the posterior end of the body. Ventral sucker 0·099 mm. across but with the space round it may appear much larger and it is difficult to define its limits. Excretory vesicle round receiving two thick lateral canals.

Here we have a very characteristic and common form but nothing further is known of its life-history. It almost certainly inhabits a bird in its adult state.

It is interesting to note that full-grown cercariae were found in the mantle cavity of *Purpura* together with the very young rediae, which seems to indicate that the cercaria emerges from its host by the same route that the redia uses to get to the digestive gland.

Cercaria patellae sp. inq.

(Lebour, 1907 B, p. 103.)

(Pl. XXIX, figs. 4, 5.)

This cercaria occurred once in the digestive gland of *Patella vulgata* from Loch Ryan out of 16 examined in September. The gonad was completely destroyed.

Redia colourless, long and transparent, 1·8 mm. or more in length. Pharynx conspicuous but no intestine could be seen. All the rediae were full of tailed cercariae.

The cercaria measures 0·50 mm. in length with the tail thin and measuring less than half the length of the body and abruptly truncated. Body covered with small spines. Head surrounded by two complete rows of longer spines. Oral sucker leads to a narrow prepharynx, muscular pharynx, long oesophagus branching into caeca reaching nearly to the posterior end of the body. Ventral sucker behind the centre of the body. Excretory vesicle receiving two unbranched lateral canals full of bright granules.

Nothing further is known of the life-history.

I had thought it was possibly a *Stephanochasmus* but it is very probably, as suggested to me by Dr Nicoll, that it is the cercaria of *Echinostephilla virgula* which I have described from the turnstone (1909, p. 3).

Genus *Stephanochasmus* Looss.

Head with two complete rows of spines. Intermediate host—fish, final host—fish.

Stephanochasmus baccatus Nicoll (I. 1907, p. 80, Lebour, 1908 A, p. 14). (Pl. XXIX, figs. 6, 7.)

The encysted form is very common under the skin of Pleuronectid fishes, the dab *Pleuronectes limanda* L., witch *P. cynoglossus* L., lemon dab *P. microcephalus* Donov., and long rough dab *Hippoglossus limandoides* Bloch, all being infected on the Northumberland Coast. Johnstone (1904, p. 98) has recorded and described it from the West Coast of England and Nicoll and Small (1909, p. 244) from Millport. The cercaria occurs beneath the skin on the under side and sometimes on the gills, the fish serving as the intermediate host. The cyst is composed of two layers, the first thick and tough, the second transparent.

The cercaria when pressed out of the cyst measures 2·9–3 mm. in length. It is pear-shaped with a very long neck, the body covered with spines which gradually disappear posteriorly. They are large near the head and surrounding the head are two rows of very long pointed spines, those in the upper row shorter than those in the lower (upper spines 0·022 mm. long, lower 0·026 mm. long). The number is variable, from 48 to 58 spines in two uninterrupted rows of 24 to 29 spines each. Nicoll thinks it probable and it seems to be very likely that we have to do with several different species. Oral sucker 0·26 mm. across, prepharynx 0·50 mm. long, pharynx 0·18 mm. long, very muscular leading to broad oesophagus branching into two coeca which reach nearly to the posterior end of the body. Genital aperture immediately in front of ventral sucker. Ventral sucker 0·23–0·26 mm. across. Two round testes and an ovary occur at the hind end of the body. Vasa deferentia joining a thin club-shaped vesicula seminalis in front of which the cirrus sac encloses a small cirrus. Oviduct short, other female organs not yet developed. Excretory vesicle very large, circular and opaque occupying the posterior region.

This cercaria is probably *Stephanochasmus baccatus* Nicoll, which he found in the halibut *Hippoglossus vulgaris* Flem. This worm has 56 spines round its head. As Nicoll suggests however there may be *S. caducus* and *S. triglae* as well as *S. baccatus* encysted in these fish and we may have examples of them all.

Other encysted Distomes have been observed but it is impossible to assign to them any position in this scheme as the younger stages are not known. These are *Cercaria limae* Nicoll and Small, *C. concava* Nicoll and Small and a cercaria which I have found frequently encysted in the mussels and cockles of Northumberland and the West Coast of Scotland, Nicoll (III. 1907, p. 91) also records an encysted Trematode from the skin, muscles, bones and layers of the eye of a *Cottus bubalis* from St Andrews, which was rendered completely blind by the parasite. He was not able to identify it. In the same work he also mentions an encysted Distomid from the gills of *Cottus scorpius* and *Gobius ruthensparri*.

I have not myself observed the following two cercariae. The descriptions are taken from those of Nicoll and Small (1909, p. 242) who kindly allow me to reproduce their figures.

Cercaria limae Nicoll and Small (1909, p. 242).

(Pl. XXIX, fig. 8.)

This was found in single isolated cysts in *Lima hians* from Millport. Cyst spherical, about 0·3 mm. in diameter, with thin opaque membranous wall. Cercaria 0·60 mm. long, elongated oval outline, colourless and transparent. Suckers globular, oral sucker 0·12 mm., ventral 0·152 mm., latter situated at the beginning of the posterior third of the body. Cuticle smooth. Two small oval testes just behind ventral sucker, their long axes directed obliquely outwards and forwards. Short excretory vesicle. Small pharynx contiguous with oral sucker. Intestinal diverticula arise immediately behind pharynx and appear to terminate near testes. The authors think this is the larval form of a *Steringophorus* or *Fellodistomum*, the latter parasitic in its adult state in the catfish *Anarrhichas lupus* and the former in various Pleuronectids.

Cercaria concava Nicoll and Small (1909, p. 243).

(Pl. XXIX, fig. 9.)

Found in a young plaice *Pleuronectes platessa* contained in a small round cyst embedded under the skin. It measured 0·17 mm. in diameter, very thin-walled so that the cercaria was easily liberated. Shape of the cercaria flattened, considerably broader towards the posterior end than towards the anterior end. Length 0·48 mm., greatest breadth 0·22 mm., oral sucker small, round, 0·06 mm. across, prepharynx 0·03 mm. long, pharynx 0·04 mm., oesophagus 0·04 mm. Intestinal diverticula bend in towards the middle line in the vicinity of the genital sucker, then curve out and again approach each other posteriorly. Excretory vesicle clearly seen, consisting of two fairly straight lateral tubes, beginning near the pharynx and running into a simple, rather wide terminal sac at the posterior end of the body. Genital sucker about the middle of the body, not a true ventral sucker. Vestigial ventral sucker in front of genital aperture. Traces of two small testes in front of the ends of the intestinal diverticula. Cuticle covered with scale-like spines.

The authors regard this as the larval form of *Cryptocotyle concava* (Crepl.) which they have found in the adult state in the shag *Phalacrocorax graculus* which feeds, as they state, largely on small plaice, dabs and flounders.

T. Scott (1909, p. 78) records an encysted Distomid from the walls of the stomach of a witch *Pleuronectes cynoglossus* from the Moray Firth. He thinks it is very probably the cercaria of *Distomum cestoides* Ed. van Beneden which he records from the oesophagus of the skate *Raia batis*, Aberdeen. He very kindly sent me specimens of the adults which are slightly different from those I have described from the starry ray *R. radiata* as *Otodistomum veliforum* (Creplin).

Cercaria mytili sp. inq. (Lebour, 1906, p. 5).

(Pl. XXIX, fig. 10.)

This occurs commonly encysted in the digestive gland of the mussel *Mytilus edulis* and cockle *Cardium edule*, chiefly in the mussel from Northumberland and the cockle on the West Coast of Scotland. The cyst is spherical and thick-walled but quite transparent, 0·20 mm. across. Very little could be made out of the structure of the cercaria as it was nearly always damaged when pressed out of the cyst.

Cercaria 0·46 mm. long, covered with spines. Posterior end pointed, head end rounded. Oral and ventral suckers about the same size 0·06 mm. across. Ventral sucker just behind the centre of the body. Alimentary system not seen. Excretory system very conspicuous in the cercaria curled up in the cyst. Two lateral canals full of clear granules.

Further life-history unknown.

(*d*) *Monostomum* group.

Cercaria ephemera Nitsch (= *Monostomum flavum* Mehlis), Lebour (1907 c, p. 436).

(Pl. XXIX, figs. 11, 12.)

This cercaria is not the larval form of *Typhlocoelum flavum* (Mehlis) as was formerly thought: the ends of the forks of the alimentary canal are not joined as they are in that genus. I have therefore returned to the name *Cercaria ephemera* given by Nitsch. It occurs in the digestive gland of *Paludestrina stagnalis* from Fenham Flats and Loch Ryan; in about six per cent. from Fenham Flats and 20 per cent. from Loch Ryan in August and November. The redia is yellowish with a dark brown intestine, well developed pharynx but no thickening round the mouth. It is active and contains cercariae in all stages.

The cercaria measures 0·24 mm. in length without its tail which is slightly longer than the body. It is opaque and speckled with brown with a brownish tinge all over the body. Anteriorly there are three conspicuous eye-spots arranged in a triangle. The body at each side of the base of the tail is drawn out and provided with a small sucker-like structure which apparently helps to fix the cercaria whilst the anterior part of the body is extended. The oral sucker is large and leads to the oesophagus which bifurcates just behind the anterior eye. There is no pharynx. The lobes of the intestine extend nearly as far as the posterior end of the body. The excretory vesicle is a clear sac at the hind end of the body with two conspicuous lateral canals full of clear granules opening into it.

The further life-history is not known but it apparently belongs to the family *Notocotylidae* Lhe., which live in the intestinal caeca and rectum of sea birds. Lühe (1909, p. 178) thinks it may be *Notocotyle triseriale* Dies. or *Catatropis verrucosa* (Fröl.), both of which live in a variety of sea birds, more particularly ducks. This species is perhaps identical with *Cercaria imbricata* Looss which he regards as the larval form of *Notocotyle triseriale* Dies. (1896, p. 192). *C. imbricata* occurs in *Bythinia tentaculata.* The usual habitat of *C. ephemera* is *Planorbis corneus* a purely fresh water Mollusk.

Cercaria lophocerca Fil. (Lebour, 1907 c, p. 443).

(Pl. XXIX, figs. 13–15.)

This peculiar cercaria occurs in the digestive gland of *Paludestrina stagnalis* from Fenham Flats (in about 40 per cent.) and Loch Ryan (15 per cent.) and also occurs in the digestive gland of the periwinkle

Littorina littorea at Millport (10 per cent.) in spring, summer and early autumn. There is a great reduction in their numbers in autumn and winter. The appearance of a periwinkle infected with it is very unhealthy, the digestive gland being a curious greyish yellow. The gonad is destroyed.

The cercaria is contained in colourless rediae with a yellowish brown intestine. The redia can be very active and constantly changing its shape. The young forms are striated transversely and have no collar nor ambulatory processes, they measure about 0·16 mm. in length. Older rediae 1·4 mm. long or longer with thickened mouth, small pharynx and conspicuous intestine. Cercaria 0·2 mm. long without its tail which may be more than three times the length of the body and provided with a very thin fin placed dorsally and ventrally for its whole length. By the aid of this finned tail the cercaria whizzes about in a most characteristic manner.

The body is covered with spines. Anteriorly are two dark eyespots one on each side of the oral sucker. Oral sucker 0·35 mm. across when at rest but it is constantly being elongated and protruded like a snout. No alimentary canal can be made out. Most of the body is occupied by two large masses of gland cells with long straight ducts running forward and opening by the oral sucker. The excretory vesicle is bilobed and oval with its long diameter transverse.

The further life-history is not known.

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DESCRIPTION OF PLATES XXV TO XXIX.

The following letters apply to all the figures.

C.	cercaria.
C.G.	cystogenous glands.
C.O.	cystogenous organs.
C.S.	cirrus sac.
E.	eye.
E.V.	excretory vesicle.
G.	gland cells.
G.A.	genital aperture.
I.	intestine.
L.C.	lateral canal.
O.	ovary.
OD.	oviduct.
ŒS.	œsophagus.
O.S.	oral sucker.
P.	pharynx.
P.PH.	prepharynx.
S.	sporocyst.
S.G.	stylet glands.
S.G.D.	ducts of stylet glands.
ST.	stylet.
T.	testis.
V.	vitellaria.
V.D.	vas deferens.
V.S.	ventral sucker.

PLATE XXV.

Figs. 1–4. *Gasterostomum gracilescens* (Rud.).
  1. Portion of sporocyst (length 1 mm.).
  2. *Bucephalus haimeanus* (length 0·3 mm.).
  3. Encysted cercaria (long axis 0·6 mm.).
  4. Cercaria pressed out of cyst (length 2·6 mm.).

Figs. 5, 6. *Gasterostomum* sp.
  5. Portion of sporocyst.
  6. *Bucephalus syndosmyae* sp. inq. (length 0·26 mm.)

Figs. 7, 8. *Gymnophallus* sp.
  7. Part of cockle from under umbo containing sporocysts with cercariae.
  8. *Cercaria margaritae* sp. inq. (length 0·2 mm.).

Fig. 9. *Cercaria scrivenensis* sp. inq. (length 0·4 mm.).

Fig. 10. *Cercaria glandosa* Lebour (length 0·2 mm.).

Fig. 11. *Cercaria macomae* Lebour (length 0·7 mm.).

Fig. 12. *Cercaria strigata* Lebour (length 0·3 mm.).

PLATE XXVI.

Figs. 1, 2. *Cercaria dichotoma* Müller.
  1. Sporocyst (length 1 mm.).
  2. *Cercaria dichotoma* Müller (length 0·2 mm.).

Figs. 3–12. *Spelotrema excellens* Nicoll.
- 3. Sporocyst (length 0·6 mm.).
- 4. Tailed cercaria (=*Cercaria ubiquita*) (length 0·12 mm.).
- 5–6. The cercaria having just entered *Carcinus maenas* and covered itself with a very thin cyst (0·014 mm. across).
- 7–11. Are various stages in the encystment of the cercaria drawn in proportion.
- 12. The cercaria pressed out of the cyst (length 0·8 mm.).

Figs. 13, 14. *Cercaria carcini* Lebour.
- 13. Cercaria bursting out of cyst (length of cyst 0·4 mm.).
- 14. Cercaria pressed out of cyst (length 0·54 mm.).

Figs. 15–17. *Cercaria balani* Lebour.
- 15. Encysted cercaria (cyst 0·4 mm.).
- 16. Cercaria pressed out of cyst (length 1 mm.).
- 17. Small cyst from *Balanus balanoides* (0·15 mm.).

Figs. 18, 19. *Cercaria corophii* Lebour.
- 18. Encysted cercaria (length of cyst 0·16 mm.).
- 19. Cercaria pressed out of cyst (length 0·34 mm.).

PLATE XXVII.

Figs. 1, 2. *Cercaria minor* sp. inq.
- 1. Encysted cercaria (cyst 0·26 mm.).
- 2. Cercaria pressed out of cyst (length 0·52 mm.).

Figs. 3–7. *Cercaria littorinae-rudis* sp. inq.
- 3. Sporocysts (1 mm. across).
- 4. Cercaria pressed out of cyst (length 0·25 mm.).
- 5–7. Different aspects of cercariae within sporocyst.

Figs. 8, 9. *Cercaria oocysta* Lebour.
- 8. Sporocyst with encysted cercariae (length 0·12 mm.).
- 9. Cercaria pressed out of cyst (length 0·24 mm.).

Figs. 10, 11. *Cercaria pirum* Lebour.
- 10. Encysted cercaria (cyst 0·08 mm.).
- 11. Cercaria pressed out of cyst (length 0·2 mm.).

Figs. 12, 13. *Cercaria linearis* Lespés.
- 12. Sporocyst (length 2·6 mm.).
- 13. Cercaria (length 0·18 mm.).

Figs. 14–16. *Cercaria brachyura* Lespés.
- 14. Young sporocyst (length 0·2 mm.).
- 15. Older sporocyst (length 1·2 mm.).
- 16. Cercaria (length (0·26 mm.).

Figs. 17, 18. *Cercaria buccini* sp. inq.
- 17. Sporocyst (length 1·4 mm.).
- 18. Cercaria (length 0·33 mm.).

Figs. 19–21. *Lepodora rachiaea* (Cobb.).
- 19. Sporocyst containing encysted cercariae (length 0·60 mm.). From a drawing by Mr Jas. Johnstone.
- 20. Cercaria pressed out of cyst (length 0·19 mm.).
- 21. Young tailed cercaria.

PLATE XXVIII.

Figs. 1, 2. *Cercaria neptuneae* sp. inq.
  1. Redia (length 2 mm.).
  2. Cercaria (length without tail 0·4 mm.).

Figs. 3–6. *Acanthopsolus lageniformis* Lebour.
  3. Young redia (length 0·3 mm.).
  4. Older redia (length 3 mm.).
  5. Young tailed cercaria.
  6. Cercaria (length 0·5 mm.).

Figs. 7–10. *Echinostomum leptosomum* Creplin.
  7. Young redia (length 0·2 mm.).
  8. Older redia (length 1·4 mm.).
  9. Cercaria (length without tail 0·5 mm.).
  10. Encysted cercaria (cyst 0·12 mm.).

Figs. 11–14. *Echinostomum secundum* Nicoll.
  11. Young redia (length 0·4 mm.).
  12. Older redia (length 2 mm.).
  13. Cercaria (length 0·7 mm.).
  14. Encysted cercaria (cyst 0·23 mm.).

Figs. 15–17. Echinostomum sp. *Cercaria littorinae obtusatae* sp. inq.
  15. Young redia (length 0·5 mm.).
  16. Older redia (length 2·5 mm.).
  17. Cercaria (length 0·78 mm.).

PLATE XXIX.

Figs. 1–3. *Cercaria purpurae* sp. inq.
  1. Young redia (length 0·32 mm.).
  2. Older redia (length 3 mm.).
  3. Cercaria (length without tail 0·64 mm.).

Figs. 4, 5. *Cercaria patellae* sp. inq.
  4. Redia (length 1·8 mm.).
  5. Cercaria (length 0·5 mm.).

Figs. 6, 7. *Stephanochasmus baccatus* Nicoll.
  6. Encysted cercaria.
  7. Cercaria pressed out of cyst (length 2·9 mm.).

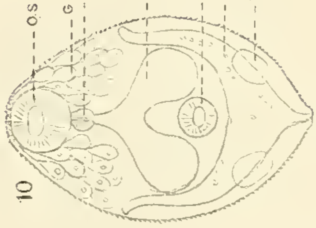
Fig. 8. *Cercaria limae* Nicoll and Small (length 0·6 mm.) from Nicoll and Small (1909, p. 242).

Fig. 9. *Cercaria concava* Nicoll and Small (length 0·48 mm.) from Nicoll and Small (1909, p. 243).

Fig. 10. *Cercaria mytili* sp. inq. (cyst 0·2 mm.).

Figs. 11, 12. *Cercaria ephemera* Nitsch.
  11. Redia.
  12. Cercaria (length 0·24 mm.).

Figs. 13–15. *Cercaria lophocerca* Fil.
  13. Young redia (length 0·16 mm.).
  14. Older redia (length 1·4 mm.).
  15. Cercaria (length without tail 0·2 mm.).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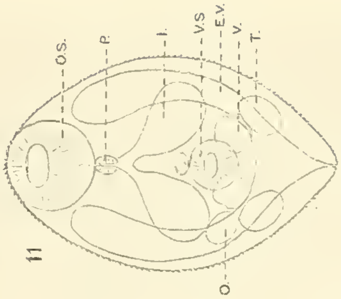
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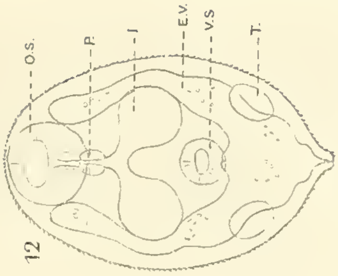
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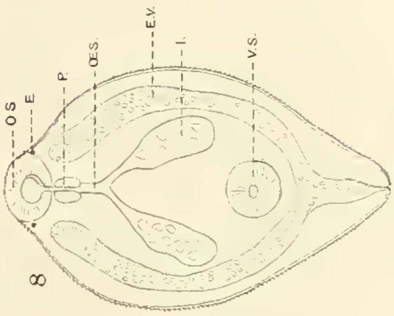
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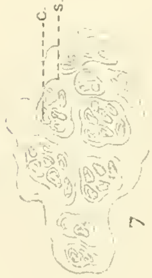
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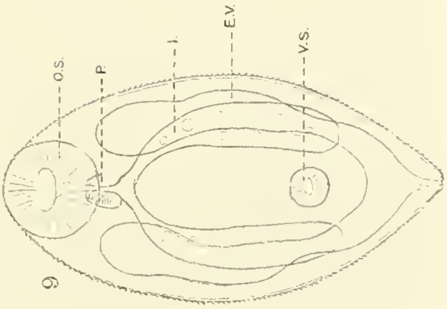
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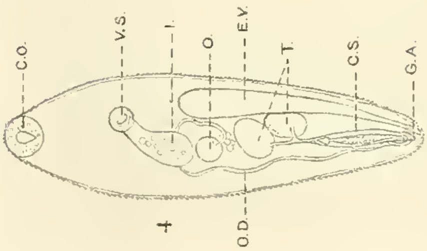
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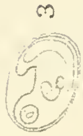
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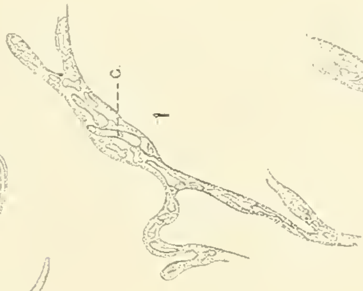
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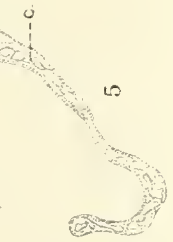
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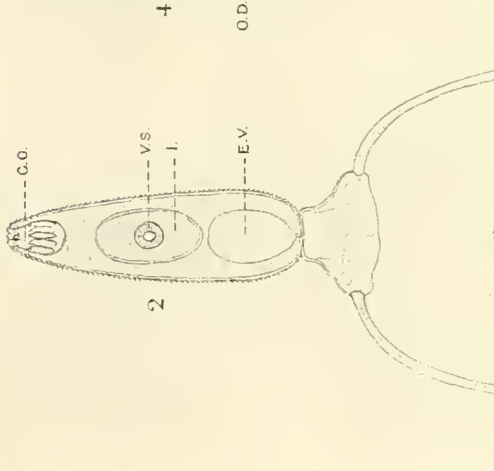
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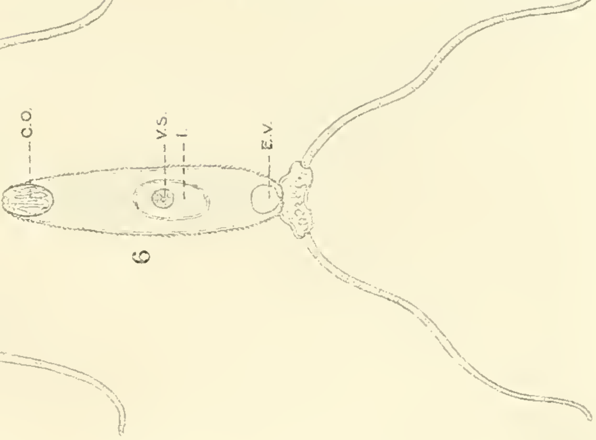
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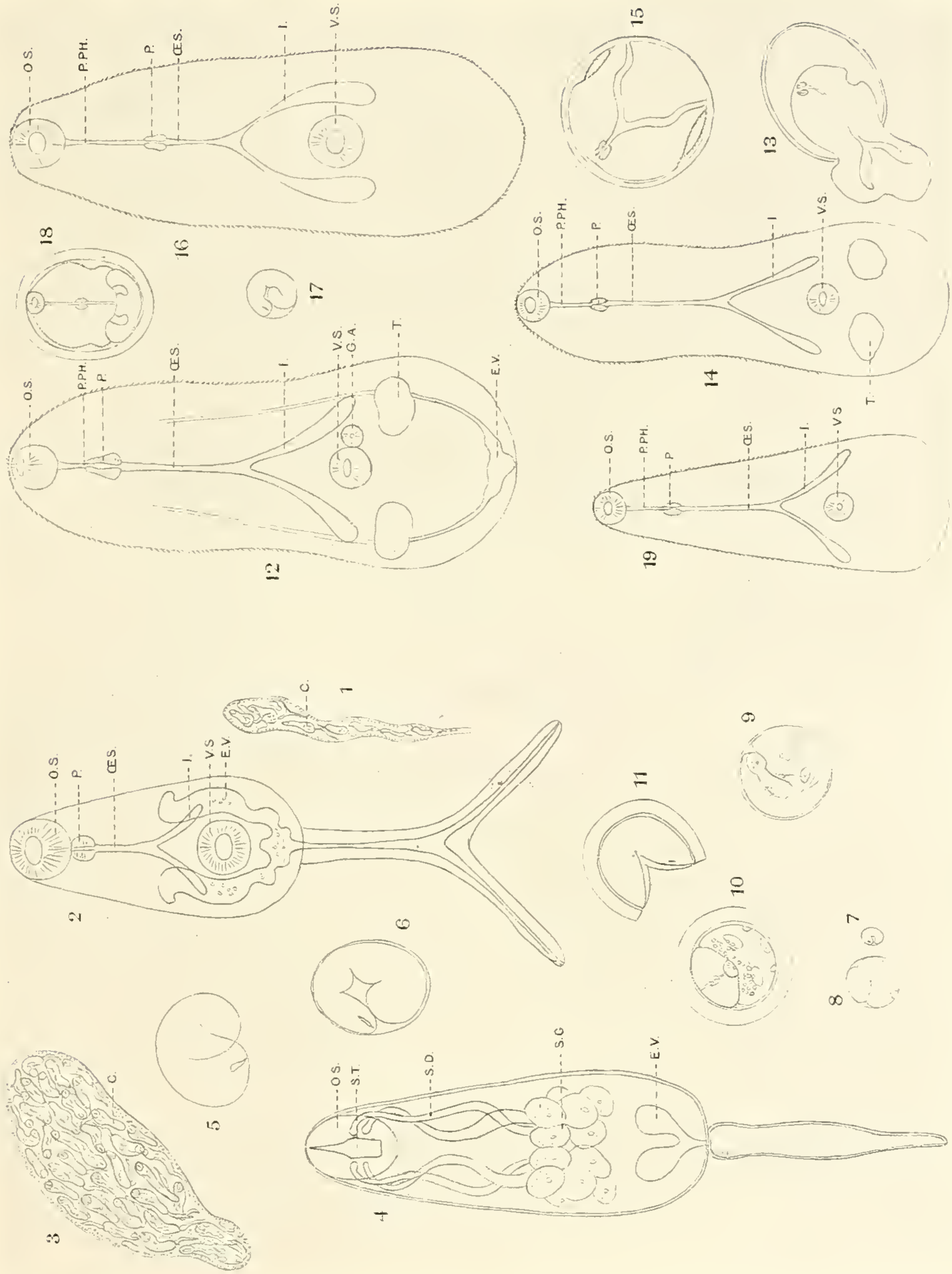
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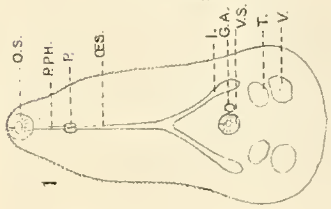

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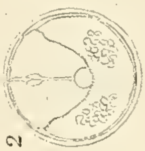
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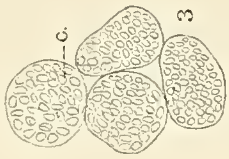

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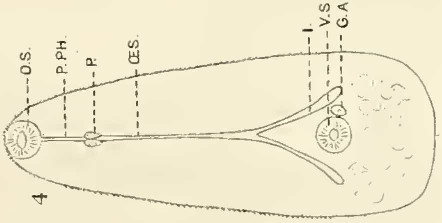

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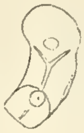
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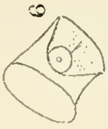
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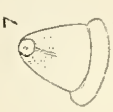
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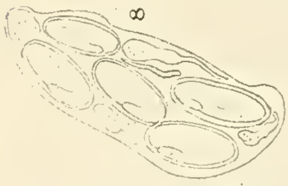
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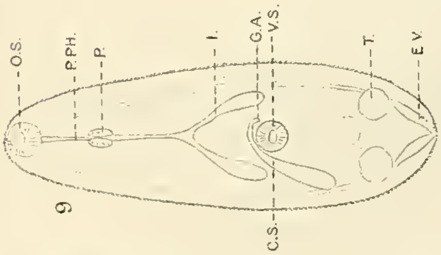

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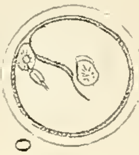
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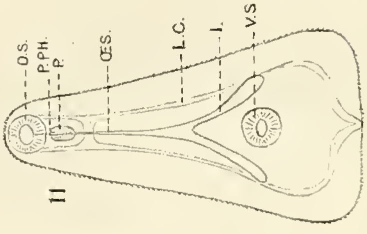

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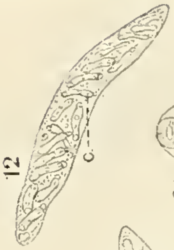

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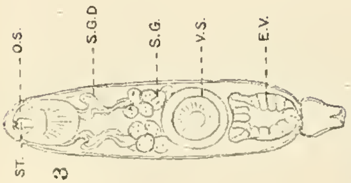

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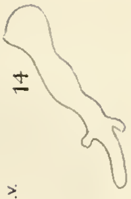
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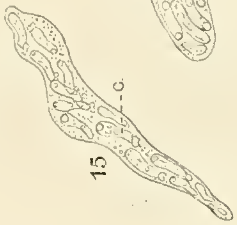

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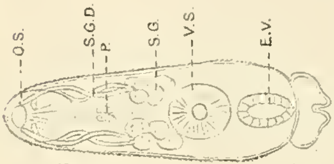

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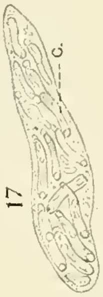

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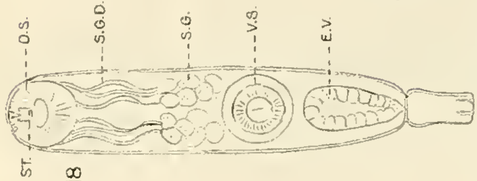

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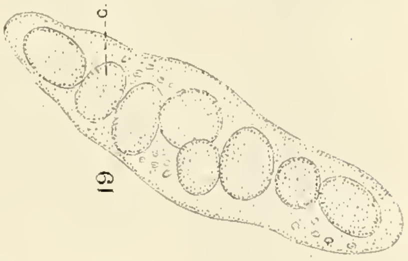

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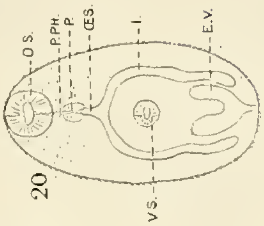

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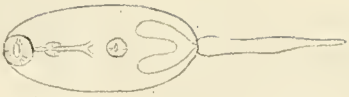
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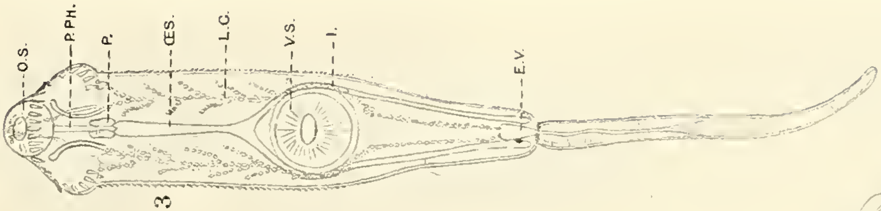
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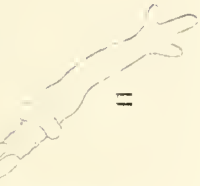
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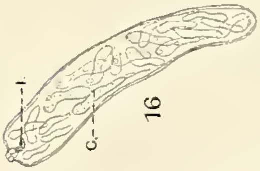
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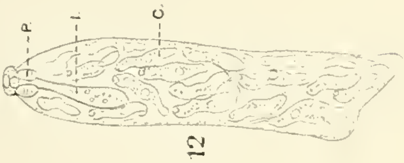
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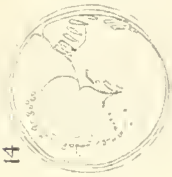
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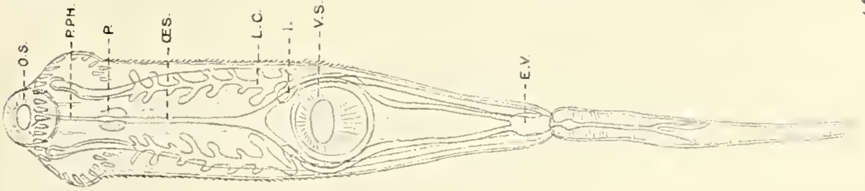
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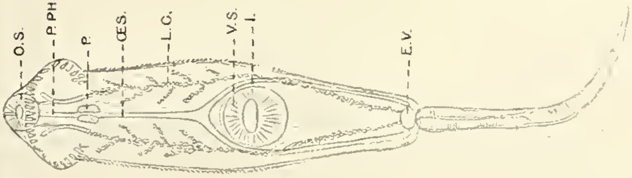
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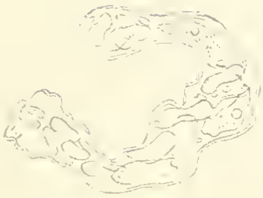
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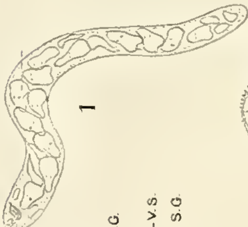
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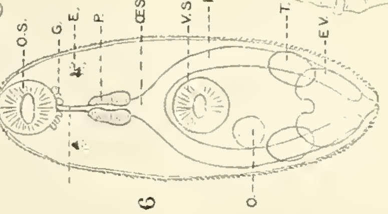
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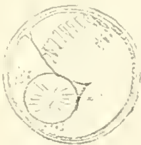
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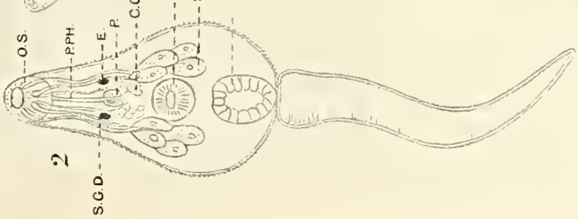
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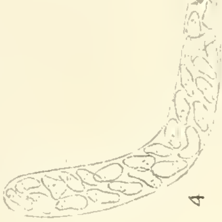
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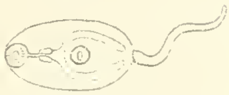
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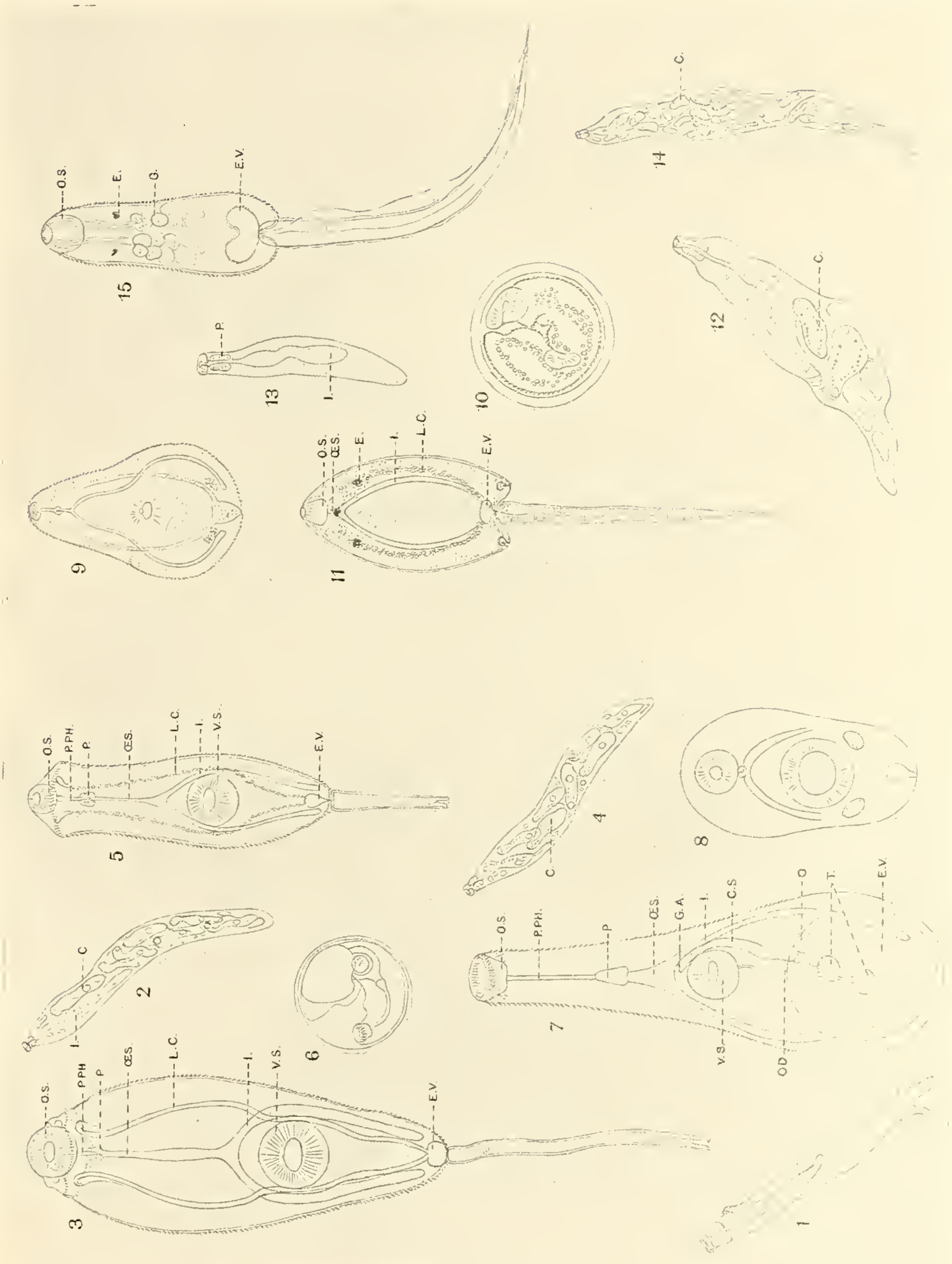
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THE KURLOFF-BODY, A SPURIOUS PARASITE.

By OTTO V. HUFFMAN, M.D.

(From the Laboratories of the Cincinnati Hospital, Cincinnati, Ohio, U.S.A.)

(With 11 Text-figures.)

Twenty years or more have passed since Kurloff first observed certain vacuoles or cell enclosures in the protoplasm of the large mononuclear leucocytes of the guinea-pig while working in Ehrlich's laboratory. Since then improved staining methods and discoveries in the field of protozoology have made it possible to take new view-points in regard to these cell enclosures which are called Kurloff's-bodies. Therefore it is not with surprise that we find Ferrata, Patella, Goldhorn, Schilling and others believing these bodies to be protozoan in nature. Patella however is the only one who claims to have observed a flagellate develop from a Kurloff-body and of course this observation would put an end to all dispute if it could be verified.

After Patella had observed a Kurloff-body develop flagella and acquire motility he undertook to prove that the Kurloff-body is an intracellular phase in the life cycle of a flagellate commonly found in an infusion of the greens which may be fed to the guinea-pigs. By isolating newly born guinea-pigs and feeding them solely upon sterilized milk and bread he succeeded in keeping the blood free of these bodies, but he did not observe his isolated guinea-pigs beyond the age of four months because they died. It is important to note along with this that Jolly and others have found that the Kurloff-body does not appear in the circulation of the guinea-pig until it is at least three or four weeks of age. Consequent upon his observation of the development of a flagellate in the blood and the results he obtained in his feeding experiment, Patella jumped to the early conclusion that the Kurloff-body is none other than the intracellular phase of a flagellate which occurs in an infusion of greens. That this was conjecture upon the part of Patella is further shown by his belief that the Kurloff-body is identical with the

Leucocytozoa (*Haemogregarina*)[1] of Bentley and Adie for he could not have arrived at such a belief if he had had any first hand knowledge of those organisms. Likewise in the controversy which ensued between him and Pappenheim it was entirely irrelevant to base any argument upon some rod-shaped bodies once observed by Auer in a case of acute lymphatic leukaemia in man, or upon a flagellated organism observed by Loewit in the blood of a patient *post mortem*.

It was Patella's observation that the Kurloff-bodies were most numerous in the blood of guinea-pigs confined in damp filthy cages, along with a similar observation of Balfour who noted that handling of the rats and dampness of the cages increased the prevalence of *Haemogregarina balfouri*, as well as the fact that the Kurloff-body is absent in the newly born guinea-pig, that led me to investigate the parasitic theory in regard to these bodies.

My studies and experiments have been carried on continuously for one year and I am greatly indebted to Professor Wherry for much assistance in pursuing the investigation. Inasmuch as the Kurloff-body was found not to be a parasite I will simply outline the results of my work.

It was my first duty to corroborate, if possible, the observations of Patella: Kurloff-bodies were patiently observed in wet blood preparations for long periods of time without the reward of seeing flagella develop. As the mononuclear leucocyte containing the Kurloff-body changes its own form or moves, the Kurloff-body undergoes a corresponding change. Several times I was surprised to see how active the Brownian movement of particles of so-called haemoconien became when in the vicinity of a Kurloff-body—but this was simply of passing interest. The flagellate which occurs in an infusion of lettuce, endive, or carrots, was found to be a species of *Bodo* and the same species was found in the contents of the caeca of all guinea-pigs examined.

With a further view to Patella's theory I examined the epithelium of the small as well as the large intestine for a possible ectoschizous or endoschizous phase of a parasite, and I likewise examined the cells of the mesenteric lymph nodes. All of the guinea-pigs contained Kurloff-bodies in the blood but in none did I find a Kurloff-body in the mesenteric lymph nodes.

My next procedure was to examine all of the organs of the body to find in which portion of the body the Kurloff-bodies occur most numerously. This was found to be in the pulp of the spleen. Here again wet preparations of blood and spleen pulp containing many Kurloff-bodies of all sizes were kept under observation for more than a month without noting any development or change in the Kurloff-bodies. The warm stage and incubator were used to preserve some of them at the same temperature as that of the guinea-pig.

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[1] For literature see *Parasitology* (1910), III. 71.

As Kurloff observed through very elaborate studies extending over several years that the large mononuclear leucocytes were not affected by splenectomy I made no effort to repeat his experiments.

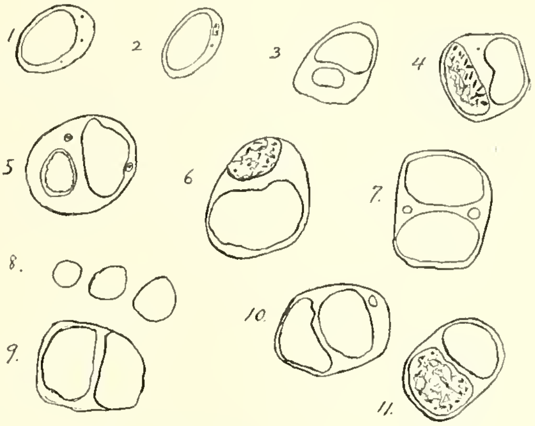
The stained preparations were made from fresh blood, spleen pulp, and from the sealed specimens which had been under observation. Various methods of fixation were tried, Schaudinn's, warm formalin vapour, ether, acids, osmic acid vapour, etc. The ordinary dry blood film fixed by methyl alcohol, dried, and stained by Giemsa gives the most uniform results; the Kurloff-body being stained a lighter shade of purple than the nucleus of the mononuclear leucocyte. If the methyl alcohol is applied to the blood before it has dried or if the Giemsa solution is applied before the methyl alcohol has entirely evaporated, the Kurloff-body may appear as a sac containing precipitated stain. If fixed with ether the Kurloff-body appears more homogeneous and stains more nearly red. The smallest bodies occur as azurophilic granules, one or more in the protoplasm of the mononuclear leucocyte and suggest an *Anaplasma*-like body. The bodies larger than a granule are stained a purplish colour; several may occur in one leucocyte and they may indent the nucleus. The most common form is about equal to the nucleus in size and is frequently associated with two or more small vacuoles in the protoplasm of the leucocyte. A distinct non-stained wall to the Kurloff-body may be evident especially when the body is smaller than the nucleus.

The fact that Kurloff-bodies occur in greater quantity in female guinea-pigs than in male caused me to make a complete study of the generative organs and their secretions but no parasites could be discovered.

So far all investigation had been based upon the assumption that the Kurloff-body is a phase of an homoïc parasite. As the ecto-parasites had never been given any consideration as possible transmitters of the supposed Kurloff-body parasite, I took as a working hypothesis that the Kurloff-body is an heteroïc parasite. It was rather disquieting to begin with to find that very lousy or flea infested guinea-pigs did not present more Kurloff-bodies in their blood than the less lousy or less flea infested. As I progressed in the work I soon found that Kurloff-bodies were present in the blood of all guinea-pigs—I never failed to find them in a guinea-pig if I continued the search for several days. I might mention here with regard to the differential counting of leucocytes from day to day that the results were quite variable, such variations being dependent upon the factors of food, the degree of heat and friction applied to the ear just before withdrawing the blood, the previous number of times the ear had been cut and how recently; but in general the proportion of polymorphonuclear forms to the mononuclear forms is about equal. The mononuclear cells containing Kurloff-bodies vary from day to day as much as from 1 to 20 per cent. of all the leucocytes.

To see what effect, if any, might result from keeping guinea-pigs free of ecto-parasites I performed the following experiment. Four guinea-pigs were thoroughly combed and brushed. Two of them were etherized and bathed in ether, alcohol, and acetic acid solution so as to kill all lice and eggs and then naphthalin was dusted and rubbed into their coat. This treatment was found unnecessary and not so good as the daily use of pyrethrum powder blown in amongst the separated hairs by an insufflator and the removing of all nits on top of the head by rubbing with a solution of 10–20 per cent. acetic acid. The guinea-pigs were placed separately in tin buckets which each day were scalded, dried, and supplied with dry sterilized saw-dust for bedding. To prevent the guinea-pigs from becoming infested with lice the buckets containing the animals were suspended from the ceiling. After a few days of such treatment I examined each guinea-pig for ecto-parasites with negative results—but to ensure the complete extermination of any lice that might have been missed an assistant was detailed to carefully examine the pigs and treat them with the powder and fresh dusty bedding every morning for several months. It was found that the removal of all lice made no appreciable difference in the number of Kurloff-bodies present. Two of the females gave birth to young under these conditions, and, while the young did not contain the larger, more mature Kurloff-bodies, they were found to harbour the smallest form of Kurloff-body, *i.e.* the azurophilic granule.

During the summer months these four guinea-pigs which were free from ecto-parasites were placed in a wet muddy hutch protected by fine wire screening and far removed from any source of infestation. Within a few days many of the mononuclear leucocytes contained from one to six azurophilic granules and within a week many of the mononuclear leucocytes contained the largest form of Kurloff-body, *i.e.* the sac, much larger than the nucleus which is crowded to one side, containing granular matter and small rod-shaped bodies. These animals upon again being placed in the dry buckets soon showed a recession in the number of Kurloff-bodies present.



Figures 1—11. *Sketched by aid of Camera Lucida.* × 1000.

Fig. 1. Mononuclear leucocyte with three azurophilic granules in protoplasm.—Fig. 2. The same with two azurophilic granules in protoplasm, one being double.—Fig. 3. Kurloff-body of moderate size, *i.e.* smaller than the nucleus of the mononuclear leucocyte which contained it.—Fig. 4. Kurloff-body with granular contents and rod-like bodies.—Fig. 5. Two small azurophilic Kurloff-bodies, and one large one which is stained purple by Giemsa and appears to have an unstained double-contoured wall.—Fig. 6. Kurloff-body, with granular contents and rod-like bodies produced by fixation before the blood film had dried.—Fig. 7. Two small vacuoles in protoplasm between nucleus and Kurloff-body.—Fig. 8. Red blood corpuscles sketched to give comparative size of leucocytes and Kurloff-bodies.—Fig. 9. Most common form of Kurloff-body, *i.e.* about the same size as the nucleus, homogeneous and stained a lighter shade of purple than the nucleus by Giemsa's stain.—Fig. 10. Vacuole in protoplasm between periphery and Kurloff-body.—Fig. 11. Last stage of Kurloff-body.

Furthermore, I made a complete study of the intestinal contents, excreta, and body juices of a large number of *Gyropus gracilis, G. ovalis, Ctenocephalus canis, Ceratophyllus fasciatus,* and *Acanthia lectularia* which had fed on guinea-pigs containing a high percentage of Kurloff-bodies. In the case of the lice and their ova, they were experimented with on the hypothesis that an organism might be liberated when the egg is ingested by the guinea-pig: sealed preparations of lice and ova mixed with gastric juice were observed; and a large number of lice were fed to a guinea-pig having few Kurloff-bodies, but nothing was discovered.

Another hypothesis suggested itself to me by finding in several instances moulds and yeasts in the spleens of guinea-pigs immediately after the removal of these organs under aseptic precautions. The fact that yeasts had been found in the spleen in cases of blastomycosis was encouraging. I thought that the Kurloff-body might be the result of the chemical activities initiated by a spore either living or after its digestion by a leucocyte. This point of view gained support from the observation that cultures made from blood alone always remained sterile whereas cultures from the spleen occasionally gave a growth of a yeast or mould.

Much of this work was repeated and carried out with much care, yet all of the results are conclusive as disproving Patella's assertion that the Kurloff-body is an intracellular stage of a flagellate.

It is important that all who experiment with guinea-pigs should be familiar with these cell inclusions which seem to be normal to the guinea-pig; for I find no less an observer than Mary Rowley, working with the assistance of the staff of the Rockefeller Institute, interpreting them as caused by the injection of blood from a case of fatal anaemia.

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ON THE LIFE-CYCLE OF *SPIROCHAETA*[1] *GALLINARUM.*

PRELIMINARY NOTE.

By E. HINDLE,
Beit Memorial Research Fellow.

(From the Laboratories of Professors Mesnil and Marchoux, Institut Pasteur, Paris; and the Quick Laboratory, Cambridge.)

(With 5 Figures and Diagram in Text.)

During the past two years I have been working on the morphology and life-history of *Spirochaeta duttoni* and, although successful in tracing parts of the life-cycle of this parasite, yet the work has been accompanied by many difficulties owing to the large percentage of ticks that are immune to infection.

Whilst working at the Institut Pasteur, Professor Marchoux kindly placed at my disposal a plentiful supply of *Argas persicus* infected with *Spirochaeta gallinarum* and, therefore, having such good opportunities of studying this parasite in both of its natural hosts, I took up the study of this closely related species and have been successful in following the main features of its life-cycle.

As, however, certain confirmatory evidence as to the true relationships of the spirochaetes is not complete and, moreover, is liable to be delayed, it has been thought advisable to publish the main results which have been obtained in the form of a preliminary note.

Two strains of *Spirochaeta gallinarum* have been employed in the following work. For the earlier observations the original strain of spirochaetosis, brought back from Brazil by Professor Marchoux, was used; later, Dr Foley sent a large number of infected *Argas persicus* from Beni-Ounif-de-Figuig, Algeria, with which most of the subsequent observations have been made.

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[1] In employing the term *Spirochaeta* instead of *Treponema* we do not thereby express disagreement with the scheme of classification proposed by Dobell (1911). This point will be considered in a later communication.

The spirochaete in the fowl.

The parasites have been examined in the living state and also in stained preparations. For fresh examination the blood film was maintained at a temperature of 40° C. by means of a thermostat and, by employing dark-ground illumination, the division of the spirochaetes could be followed through all its stages.

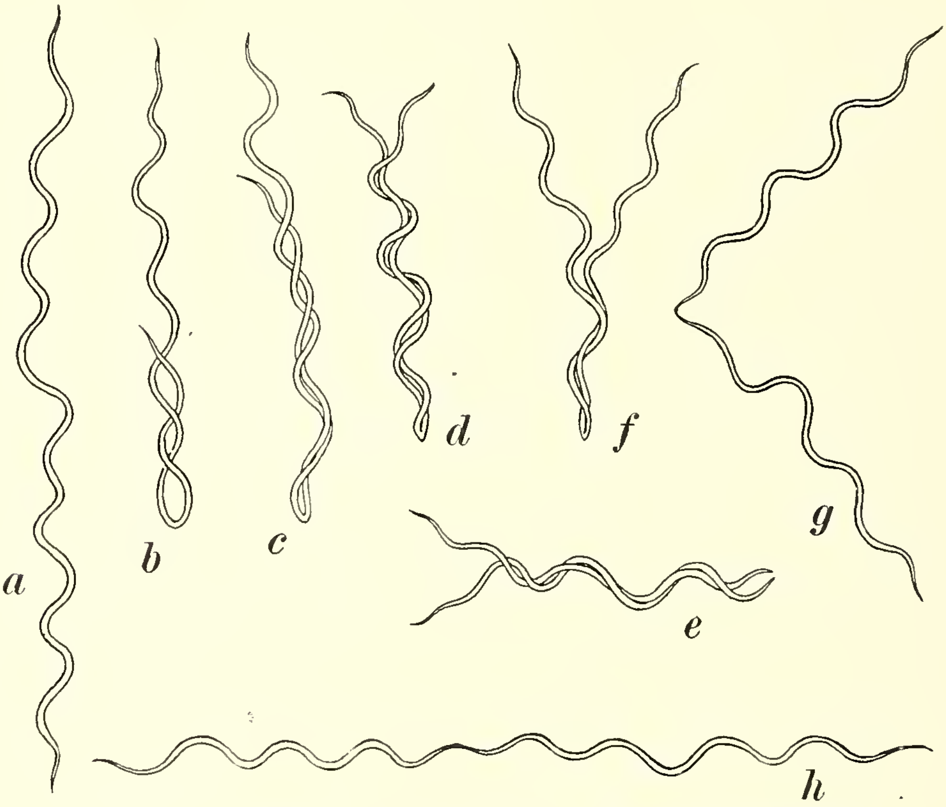

Fig. 1 *a–h*. Various stages in the flexion and transverse division of *Spirochaeta gallinarum*.

The details of division seem to differ in some respects from that previously described for blood spirochaetes (*e.g.* Mackinnon, 1909; Fantham and Porter, 1909), but resemble that of *Cristispira*, described by Gross (1910), and *Spirochaeta anodontae*, described by Bosanquet (1911).

The various stages are shown in the accompanying text figure (Fig. 1). One end of a long spirochaete (*a*) doubles back (*b*), the reflexed portion being closely applied to the main part of the parasite. The reflexed part gradually increases in length (*c*), all the time being closely wound round the other portion of the spirochaete (*b*, *c*), and thus the two ends approach each other and finally appear in juxtaposition (*d*). The appearance now presented by the parasite is that of two spirochaetes tightly coiled together, except that at one end of the coil the two threads are continuous, being flexed through an angle of 180°.

After having passed through this process, which takes place with considerable rapidity, the two halves of the spirochaete uncoil from each other (*f*, *g*) and separate at the point of flexion (*g*), thus producing two parasites. Sometimes the spirochaete breaks in two, before the daughter parasites have uncoiled from each other (*e*). In the former case the parasites may remain connected together for some time before finally separating (*h*) and in this case exactly resemble the forms of transverse division previously recorded for these parasites.

As the two halves of the dividing spirochaete are very closely wound together before separating, the process of unwinding and final separation into two halves simulates longitudinal division to an extraordinary degree and, in many cases, it required the most careful examination to decide whether one was dealing with a case of true longitudinal division, or the above described transverse fission. I have never observed longitudinal division in either this species or *S. duttoni*, and it seems probable that the so-called "longitudinal fission" might be explained as the final stage of this peculiar method of transverse division. As for those cases in which an apparently single, long spirochaete merely separates into two approximately equal halves, it is impossible to say whether they represent the last stage of the above-described division, or merely the separation of two distinct parasites which have been temporarily agglutinated together by their ends. It may also represent direct transverse division without any previous flexion of the spirochaete.

In any case, whatever may be the true interpretation of the latter forms, the division of *S. gallinarum* is certainly usually transverse, and as I have devoted considerable time to the observation of the parasite without ever having observed any sign of longitudinal fission, I believe that it is invariably transverse.

By means of examination with the dark-ground illumination, I have frequently observed the breaking up of the spirochaete into a number of coccoid forms (? spores), in the manner described by Balfour (1911) for this species, and also by Bosanquet (1911) for *S. anodontae.* I can entirely confirm Balfour's description of this interesting process, which takes place at the crisis of the disease or after drug treatment.

It is observed much more frequently in the contents of the gut of an *Argas*, which has previously fed upon an infected bird. The spirochaete gradually assumes the appearance of a chain of beads (Fig. 2 *a–d*) contained within the transparent cell-wall.

After swimming about for some time in this form, the spirochaete appears to rupture at one end and the coccoid bodies escape into the surrounding medium, leaving an empty sheath behind them (*e*). In some cases the whole cell-wall seems to disintegrate before the coccoid bodies escape, but the final result is the same, viz. the liberation of a varying number of minute round or ovoid bodies (*f*).

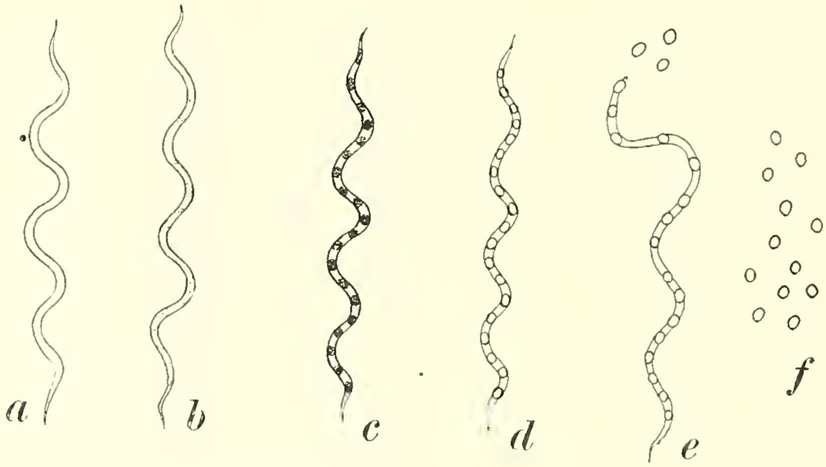

Fig. 2 *a–f*. Successive stages in the formation of the coccoid bodies (diagrammatic).

The fact that these coccoid forms are produced in large numbers after drug treatment, or when the parasites are in unfavourable conditions, has caused them to be regarded previously as merely the result of granular disintegration. Apart from their regularity in size and appearance, which in itself precludes the possibility of their being regarded as degeneration products, the time of their appearance is just when one would expect them. Moreover, the appearance of degenerating spirochaetes is very different from that presented by segmenting forms, as, instead of the production of a row of coccoid forms of uniform thickness, either the whole spirochaete gradually dissolves away, or, as the result of plasmolysis, one or more irregular swellings are formed along the length of the parasite (Fig. 3 *a–e*).

Some of these degeneration forms are very peculiar and have given rise to the belief in the existence of male, female and indifferent forms, which still exists among certain authors. One form of degeneration that is particularly common is the production of a large round cyst-like body about the middle (*a–d*), or at one of the extremities of the spirochaete (*e*). This is the result of the cell membrane of the parasite becoming swollen out like a bubble, as a result of the pressure of the contents. These forms have been repeatedly described as "cysts," but they are merely one of the results of plasmolysis.

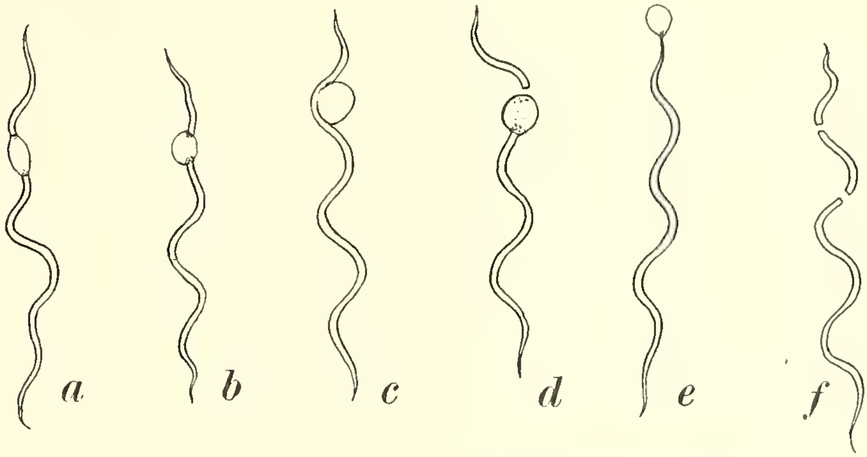

Fig. 3 *a–f*. Degeneration forms, showing the production of large cyst-like bodies.

The examination of stained preparations has not added much to the information derived from a study of the living parasites. In most cases the films were fixed in osmic acid vapour, then hardened in absolute alcohol and stained either with Giemsa, or one of its modifications. Some films were fixed in Flemming's solution, or in sublimate alcohol and then stained with Heidenhain, or safranin and methylene blue (Hindle, 1909), the films never being allowed to dry. These latter preparations were of use as confirmatory evidence of the results obtained by a study of dried films.

The examination of the spirochaetes, no matter what method of staining was employed, has not revealed the existence of any differentiation in the contents of the cell. Neither chromatic granules nor anything even suggesting a nucleus can be seen and the whole parasite appears to consist of a thick cell-wall (periplast) surrounding a homogeneous core which, from its staining reactions, seems to consist of chromatic material.

At certain periods, especially after being ingested by the tick, the endoplasm of the spirochaete breaks up into a series of darkly staining bodies (Fig. 2 *c*), which become rounded off in the form of minute coccoid bodies or spores, contained within the cell-wall (Fig. 2 *d*). These coccoid bodies then escape by rupture of the cell-wall and appear in the plasma as round, or ovoid, chromatic-staining bodies, the diameter of which is usually about 0·2 μ. The true nature of these bodies is problematical, for although in some respects they resemble the spores of bacteria—especially the *Disporea*—in their formation, yet the fact that they stain deeply and also multiply (see below), at once differentiates them from true spores. From their shape and subsequent behaviour, it is preferred to call them "coccoid bodies" and their nature will be discussed in a future communication.

The development of these coccoid bodies into spirochaetes has not yet been observed in the blood of the fowl, as the spirochaetosis of fowls rarely assumes the relapsing type. It has been followed, however, in the body of the tick and will be described under that section.

I have not been successful in observing the penetration of the spirochaetes into the red blood corpuscles in the manner described by Prowazek (1906), but from their behaviour in the tick it is quite possible that it may occasionally take place. The transformation of spirochaetes into the intracorpuscular bodies of Balfour (1909) has, therefore, not been observed, although these bodies have been met with occasionally.

A careful examination of a number of normal fowls has revealed the fact that occasionally intracorpuscular bodies, identical in appearance with those described by Balfour, may be found in the red cells, and consequently it is extremely probable that they are the products of nuclear degeneration. The fact that the nucleus, or its contained nucleolus, may bud off fragments into the cytoplasm is well known and also occurs in the red cells of normal fowls. That the appearance of numbers of these bodies is associated with an attack of spirochaetosis is no more significant than the fact that haemolysis accompanies an attack of piroplasmosis. It is known that nuclear degeneration of certain cells is associated with some diseases (*e.g.* rabies; Acton and Harvey, 1911) and, therefore, there is nothing improbable in the view that the intracorpuscular bodies of Balfour are of this nature. It is important to note that Balfour's figures show that these bodies have exactly the same staining reaction as the nucleus of the red cell, and, moreover, are identical in appearance with the products of undoubted nuclear degeneration seen in normal fowls.

This nuclear degeneration seems to be a special feature of the fowl spirochaetosis occurring in the Sudan and in South Africa (Jowett), for it only rarely occurs in the blood of fowls infected with the strains of this disease from Brazil and Algeria respectively. With these latter strains of spirochaetosis the infected bird usually dies at the height of infection, or else recovers, and the infection rarely assumes the chronic form, necessary for the production of abundant nuclear degeneration of the red cells.

The spirochaete in the tick.

After being taken into the tick, some of the spirochaetes penetrate the wall of the gut and appear in the coelomic fluid. The time which elapses between the ingestion of the parasites and their appearance in the coelomic fluid varies from 2 hours to as long as 48 hours and, moreover, their appearance is by no means constant, for in some cases we have never seen any spirochaetes in the coelomic fluid, although the tick was examined at intervals for four days after having fed on an infected fowl. Usually the parasites only remain in the coelomic fluid a short time, as they bore their way into the salivary glands and gonads of the tick within a few hours.

As a result we have found numerous spirochaetes in the salivary glands and ovary, respectively, of a tick that had fed on an infected bird 6 hours previously. As this tick had been kept at a uniform temperature of 28° C. for at least three months previously, the spirochaetes in these organs must have come from the gut. Contamination during the dissection certainly did nor occur in this case, for the preparations show a complete absence of any of the characteristic gut-contents.

The spirochaetes in these organs and also in the Malpighian tubules bore their way into the cells (Diagram A), and after becoming more or less coiled up, often producing cyst-like forms, segment into a number of "coccoid bodies." These intracellular coccoid bodies multiply by transverse fission, especially in the cells of the Malpighian tubules and the ovary. As a rule they soon disappear from the salivary glands and therefore it seems probable that, as in the case of *O. moubata* (Leishman, 1910; Hindle, 1911), these organs are not usually responsible for the infection, but that it is produced by the excretion of infective material which enters the open wound caused by the tick's bite.

In support of this view I might mention that, although the majority of *Argas persicus* do not emit their coxal fluid and excrement whilst attached to the fowl, yet a few were noticed to pass their secretion immediately after feeding. In these cases the excreted substance bathed the open wound caused by the tick's bite. The injection into a Java sparrow[1] of some of this material—coxal fluid mixed with excrement—from one tick was followed by a severe spirochaetal infection, and yet, in practice, it is generally necessary to feed numerous ticks on a bird in order to produce an infection. I believe that the reason of this latter fact is that, as a rule, only those few *Argas* which emit their coxal fluid and excrement whilst on the bird are responsible for the infection. The fact that spirochaetes may be found in the salivary glands is evidence that these organs may be infective, but as both the spirochaetes and the resulting coccoid bodies often disappear from this part of the tick, it is evident that it is not a favourable situation for their development.

Many of the spirochaetes remain in the lumen of the gut and it is in this situation that one observes the formation of the coccoid bodies with the greatest clearness. One to two days after the parasites have been ingested into the gut of the tick, by examination with dark-ground illumination, large numbers of spirochaetes may be observed undergoing this process of the formation of the coccoid bodies. As a result the spirochaetes rapidly disappear from the gut of *Argas* (in three to ten days, according to the temperature), whereas, when infected blood is merely kept in a sealed glass tube, the parasites may persist for as long as three months and show various stages of degeneration, some of which have been described above (Fig. 3).

The resulting coccoid bodies persist in the lumen of the gut and Malpighian tubules and are excreted together with the Malpighian secretion. When the crystals escape from the Malpighian tubule the intracellular coccoid bodies may also become free and thus the Malpighian secretion is continually infected, and when mixed with the coxal fluid it may enter the open wound caused by the tick's bite. The coccoid bodies would then be able to develop into spirochaetes in the blood of the bird, although this process has not actually been followed.

The development of the intracellular coccoid forms into normal spirochaetes and also into fusiform bacilli has been repeatedly observed in the tick. If an infected *Argas* be kept at a temperature of 37° C., after about five days spirochaetes appear in the coelomic fluid, the lumen of the gut, and all the organs of the tick. By making a series of films commencing with the unincubated tick and ending with those containing fully developed spirochaetes, all the stages in the development of the latter forms from the coccoid bodies may be obtained.

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[1] These birds are not more susceptible to *S. gallinarum* than fowls. From their small size they are more convenient for inoculation experiments.

The effect of heat on the development of the intracellular coccoid bodies is rather variable and does not always result in their development into spirochaetes. The first effect seems to be the production of growth and transverse fission, resulting in the production of large masses of coccoid bodies that simulate schizogony. Subsequently, certain of the coccoid forms elongate, first assuming a bacillus form, and then appearing as short spirilla (Fig. 4). Some of the bacillus forms merely elongate into long fusiform bacilli which are usually intracellular. The short spirilla escape from the cells into either the lumen of the gut and Malpighian tubules, or into the coelomic fluid, and there grow in length until they appear as normal spirochaetes (Fig. 4). The various stages in their elongation up to the normal spirochaete have been repeatedly observed in the tick and, therefore, it may reasonably be assumed that the same form of development occurs when the coccoid bodies are introduced into the blood of a fowl. As the spirochaetes appear in the coelomic fluid of a tick which has been incubated at 37° C. for a few

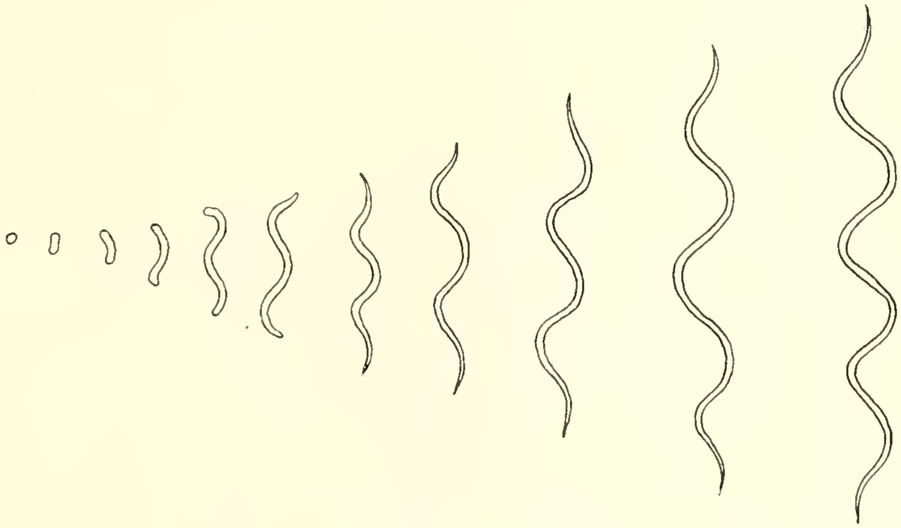

Fig. 4. Stages in the development of spirochaetes from the coccoid bodies.

days, the parasites naturally make their way from this medium into all the organs, where they may easily be found.

The development of the coccoid bodies was also followed in the eggs laid by infected *Argas*. It was found that the eggs usually contain a considerable number of these coccoid bodies and as in this case there is absolutely no possibility of external contamination, many were kept at a temperature of 37° C. in order to follow the development of the spirochaetes. This was all the more important as the only experiments with the larvae hatched from the eggs laid by an infected *Argas*, seemed to show that the infection was not transmitted to the larvae. Blanc (1911) has used this as an argument against the view so ably put forward by Leishman (1910) that the granules (= coccoid bodies) represent a stage in the life-history of the spirochaete.

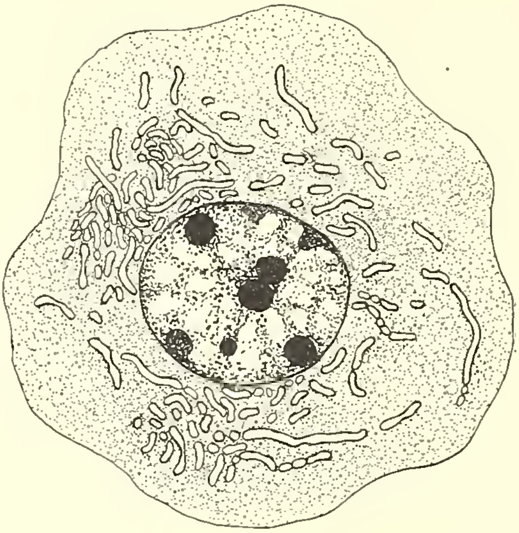

Fig. 5. Cell from the embryonic Malpighian tubule of an egg that has been heated for 5 days at 37° C. Showing the elongation of the intracellular coccoid bodies resulting in the production of long filaments.

It was found, however, that not only could spirochaetes be obtained by maintaining the eggs containing coccoid bodies at a temperature of 37° C., but that also infection could be produced by feeding the infected larvae on a fowl. A large number of larvae, hatched from eggs laid by infected *Argas* from Algeria, were placed on a fowl. The bird showed parasites four days later and the next day died from the infection, with its blood swarming with spirochaetes. Another batch of larvae hatched from the eggs of the *Argas* from Brazil, but possibly mixed with some of the Algerian strain, also produced a fatal infection in a fowl. With the first stage nymphs obtained from these larvae another fowl has also been infected and we are rearing these nymphs in order to see whether the infection is transmitted to their offspring, as in the case of *O. moubata*.

The study of the coccoid bodies in the developing eggs is of great interest for these forms seem to concentrate into particular regions of the embryo. The cells of the Malpighian tubule when in the embryo often contain enormous masses of the coccoid bodies, which, in some cases, are so numerous as to completely fill the cells. In this respect they agree with the similar stages of *S. duttoni* occurring in the developing eggs of *O. moubata* (Leishman, 1910). If the developing eggs were kept at a temperature of 37° C. either long fusiform bacilli or spirochaetes were obtained, according to the time at which one commenced the incubation.

If the eggs were placed in the incubator as soon as they had been laid the larvae did not hatch out until the 7th or 8th day and in the films made from them the simple elongation of the coccoid bodies into intracellular, long fusiform bacilli was observed (Fig. 5). No spirochaetes were found in the films made from the freshly hatched larvae, nor, in fact, from those made from the larvae after they had been kept in the incubator for another three or four days. Moreover, in these larvae the coccoid bodies and fusiform bacilli seemed to disappear after the 7th day.

On the other hand two batches of infected eggs that were allowed to develop at 28° C. for about a week before being placed in the incubator, both showed abundant spirochaetes in the larvae that hatched from them about four days later. In this case the various stages in the development of the coccoid forms into normal spirochaetes were the same as those occurring in the incubated adult tick.

It seems possible, from a consideration of the above-described results, that whilst the coccoid forms are intracellular they can either multiply by transverse fission or simply elongate into fusiform bacilli or short spirilla, identical in appearance with ordinary bacteria. In order to develop into spirochaetes it is necessary for them to escape from the cell into a fluid medium ; in the case of the tick, either the coelomic fluid, or the contents of the gut. When the eggs are allowed to develop at 28° C. for a few days before being placed in the incubator, the developing spirochaetes are able to escape from the cells into the coelomic fluid, which is not formed until late in the development of the larvae. As a result, in this fluid medium they grow up into normal spirochaetes resembling those occurring in the blood.

The inoculation of these coccoid bodies into Java sparrows has not always been followed by infection and there is evidently some other factor in their development which is not quite clear. One experiment has been made, however, which suggests that the coxal fluid may be of assistance in causing the development.

A number of eggs containing numerous coccoid bodies were crushed up and the resulting material divided into two halves. One part was mixed with some coxal fluid, carefully collected to avoid any possibility of it being contaminated by excrement, and the mixture then injected into a Java sparrow. The other half of the crushed eggs were directly injected into another sparrow. The first bird became infected five days later and died of the infection with its blood swarming with spirochaetes, whereas the second bird never became infected.

On another occasion the injection of eggs containing coccoid bodies that had been heated at 37° C. for 36 hours produced a very slight infection in a Java sparrow. Two injections of the coccoid bodies from eggs that had been heated at 37° C. for 24 hours were followed by negative results, whilst on another occasion an injection of similar material produced a slight infection. The results, therefore, are somewhat contradictory and show that there is still some undetermined factor connected with their development.

It is possible that when the coccoid bodies mixed with the coxal fluid enter the wound caused by the tick's bite, the spirochaetes multiply at the site of infection before entering the general circulation.

Up to the present we have been unable to cause the development of these coccoid bodies in culture media, but there is little doubt that they are capable of being cultured, for both Tunnicliff (1906) and Noguchi (1911) have succeeded with other species of spirochaetes. The former, working with *S. vincenti*, obtained cultures of the fusiform bacillus forms, which, in the case of *S. gallinarum*, are obtained in the cells of *Argas* that have been heated at 37° C. for a few days.

Therefore, it is possible that one of the stages of the spirochaete may be cultured without the spirochaete form being developed.

The discussion of the true position of the spirochaetes will be postponed until a more detailed account of this parasite is published.

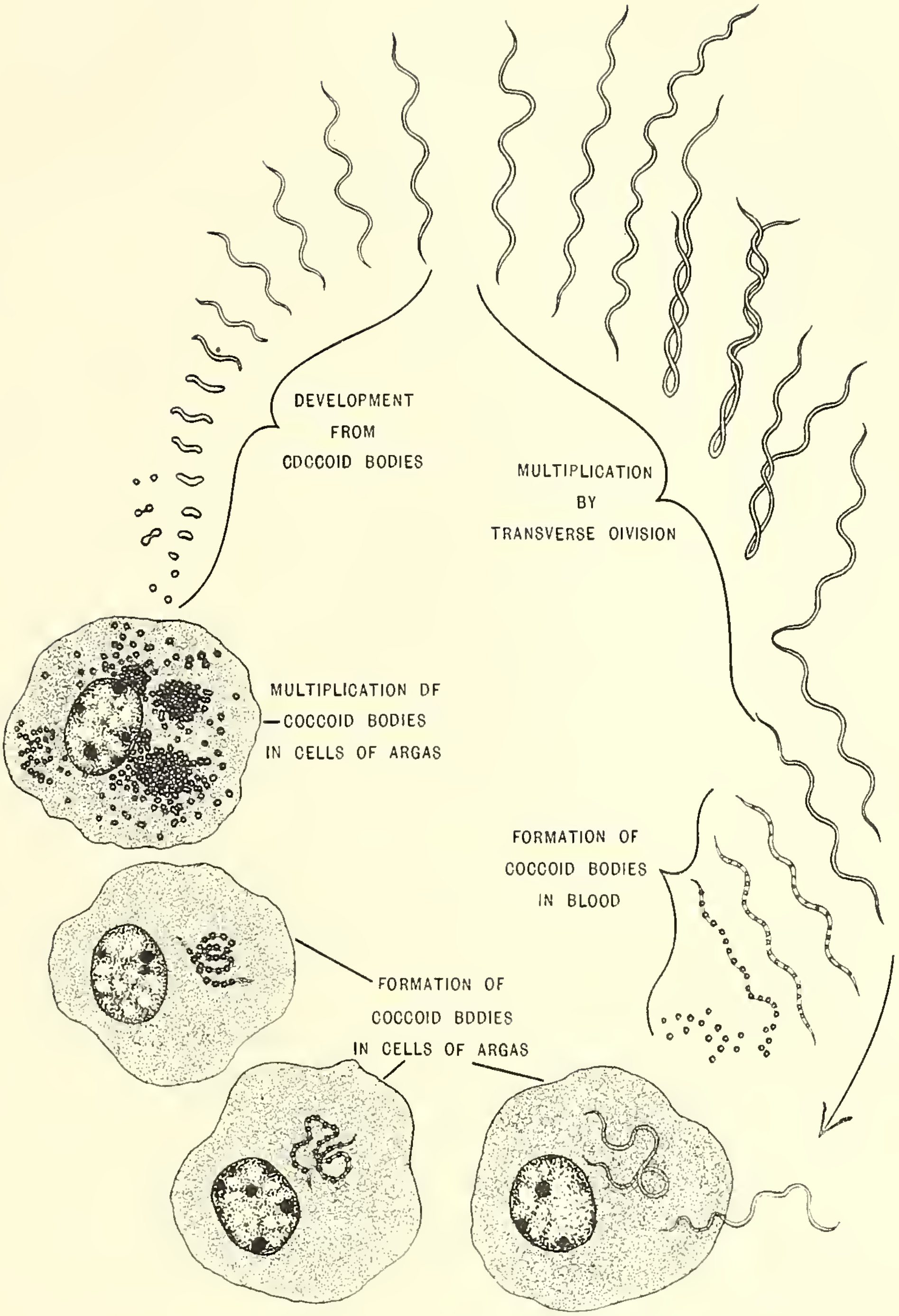

Diagram *A*. The life-cycle of *Spirochaeta gallinarum* (diagrammatic).

SUMMARY.

The life-cycle of *S. gallinarum* may be briefly summarised as follows:

Commencing with the ordinary parasite in the blood of the fowl, the spirochaete grows until it reaches a certain length (16 μ–19 μ) and then divides by the peculiar mode of transverse division described above. This process is repeated and is probably the only method of multiplication of the parasite within the blood. When the spirochaetes disappear from the circulation some of them break up into coccoid bodies which, however, do not usually redevelop in the fowl. When the spirochaetes are ingested by *Argas persicus*, some of them pass through the gut wall into the coelomic fluid. From this medium they bore their way into the cells of the various organs of the tick and there break up into a number of coccoid bodies. These intracellular forms multiply by ordinary fission in the cells of the Malpighian tubules and gonads. Some of the coccoid bodies are formed in the lumen of the gut and Malpighian tubules. The result is that some of the coccoid bodies may be present in the Malpighian secretion and excrement of an infected tick and when mixed with the coxal fluid may gain entry into another fowl by the open wound caused by the tick's bite. They then elongate and redevelop into ordinary spirochaetes in the blood of the fowl, and the cycle may be repeated.

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NEW SPECIES OF TICKS (*HAEMAPHYSALIS, AMBLYOMMA*).

By L. E. ROBINSON, A.R.C.Sc. Lond.

(*From the Cooper Laboratory for Economic Research, Watford.*)

(With 4 Text-figures.)

The following descriptions relate to two new species of the genera *Haemaphysalis* and *Amblyomma*, respectively, and to a new variety of *Amblyomma variegatum*:

Haemaphysalis silacea sp. n. ♀, from South Africa (370)[1].
Amblyomma variegatum var. n. *nocens*, ♂, from South Africa (371).
Amblyomma fiebrigi sp. n. ♂ and ♀, from South America (6).

Haemaphysalis silacea sp. n.

Fig. 1, *a–f*.

Male: unknown.

Female (ungorged): *body* 2·4 × 1·7 mm., ovate, narrowing anteriorly; colour earthy yellowish-brown; dorsum punctate, almost glabrous. Marginal groove commencing some distance behind the scutum and terminating at the antepenultimate festoon. Postero-median and accessory grooves represented by broad shallow depressions; festoons well-defined. *Scutum* 1·04 × 1·17 mm., broadly ovate, almost circular; colour somewhat darker than the general body colour; deeply emarginate, scapulae rounded; punctations fine, fairly numerous and regularly distributed. Cervical grooves of moderate depth, slightly convergent posteriorly. *Capitulum* (*l.* 440 μ) short and broad; basis capituli rectangular, about three times as broad as long; cornua short, with rounded extremities; porose areas well separated, the interval as great as or exceeding the double diameter. Palps hemicylindro-conical, with no prominent lateral protrusion; articles 2 and 3 with postero-ventral, -lateral and -dorsal margins slightly salient; article 3 with a retrograde process on the ventral surface only. Hypostome dentition 4 | 4. *Venter* scattered with pale hairs; genital orifice opposite inter-space between coxae II and III; genital grooves widely separated and

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[1] These figures refer to numbers in W. F. Cooper's collection.

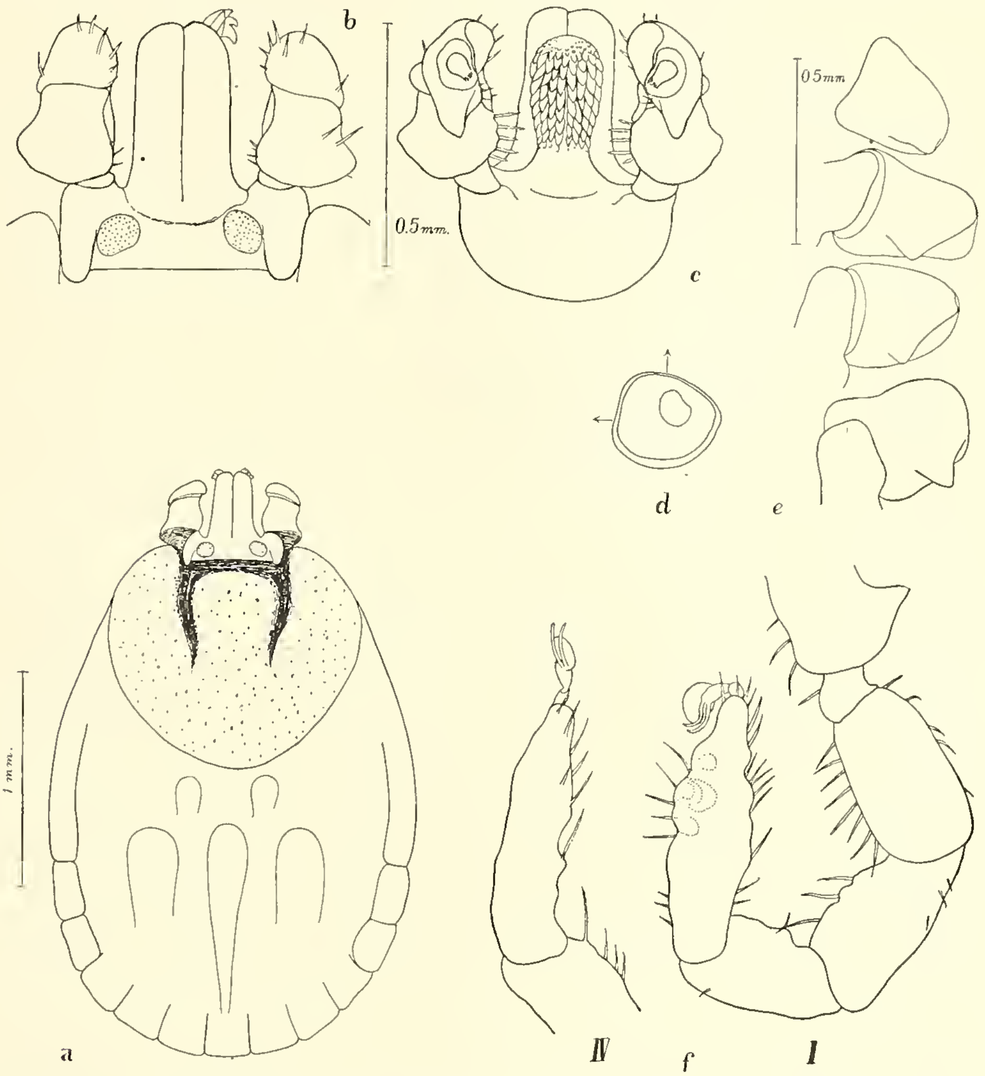

Fig. 1. *Haemaphysalis silacea* ♀ : (*a*) dorsum (capitulum foreshortened); (*b*) capitulum, dorsal aspect; (*c*) capitulum, ventral aspect; (*d*) spiracle (the arrows indicate the anterior and lateral margins, respectively); (*e*) coxae I–IV; (*f*) tarsi I and IV. *f*—same magnification as *b* and *c*, *d*—same magnification as *e*. (C. 370. L.E.R. del.)

divergent; spiracles almost circular (270–290 μ). *Legs*: coxa I with a small wide triangular spur, coxae II and III with an oblique salient ridge; coxae IV with a wide triangular spur, larger than that on coxa I. Tarsi tapering gradually, unarmed; tarsus IV more than three times as long as broad.

Origin: Gonubie Park, East London, South Africa. Described from 4 ♀'s found on oxen allowed to run on "starvation camp" from which stock had been excluded for two years.

Amblyomma variegatum var. n. **nocens.**

Fig. 2, *a–f*.

Male: *body* (*l.* 4·6–5·5 mm., *w.* 3·6–4·2 mm.) closely resembles that of the type. *Scutum*: ornamentation[1] differs slightly from that of *A. variegatum*; the pale areas anterior to the falciform stripe are golden-yellow in colour; those posterior to the falciform stripe, together with the lateral pale areas, coppery-red narrowly margined with green; falciform stripe isolated; cervical stripes may or may not extend backwards to fuse with the falciform stripe; lateral spots conjoined, forming an arch, or indistinguishably fused with the dark-coloured marginal band, the lateral pale areas in such a case being absent; postero-median stripe narrow, with a slightly knobbed extremity; accessory stripes narrower than those of the type, directed towards the postero-lateral angles of the falciform stripe; margins and festoons entirely dark-coloured. The dark markings are more elevated above the general surface than is the case in the type. Cervical grooves deep. Punctations numerous, much coarser than in the type, confluent in places. Eyes hemispherical, pale or dark, orbited. *Capitulum* typical. Palps slender, contracted at base of article 2; article 1 carinate on ventral surface. Hypostome long, dentition 3|3 on greater portion, 4|4 on proximal portion. *Venter* typical, spiracle short comma-shaped. *Legs* typical; coxae I with two stout spurs, the internal blunt, the longer external spur more pointed; coxae II and III with a salient ridge, three-fourths the width of the coxa; coxae IV with a single stout spur.

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[1] The descriptive nomenclature of the ornamentation used above, is based on that prescribed by Dönitz, W. (1909), "Über das Zeckengenus *Amblyomma*," *Sitzungsber. d. Gesellsch. naturforsch. Freunde*, No. 8, and "Die Zecken Südafrikas," *Schultze, Forschungsreise*, IV. (3), *Jenaische Denkschr.*, XVI.

Falciform stripe = *Sichelfleck*, cervical stripe = *Cervicalstrich*, lateral spot = *Seitenfleck*, postero-median stripe = *Mittelstrich*, accessory stripe = *Nebenstrahl*.

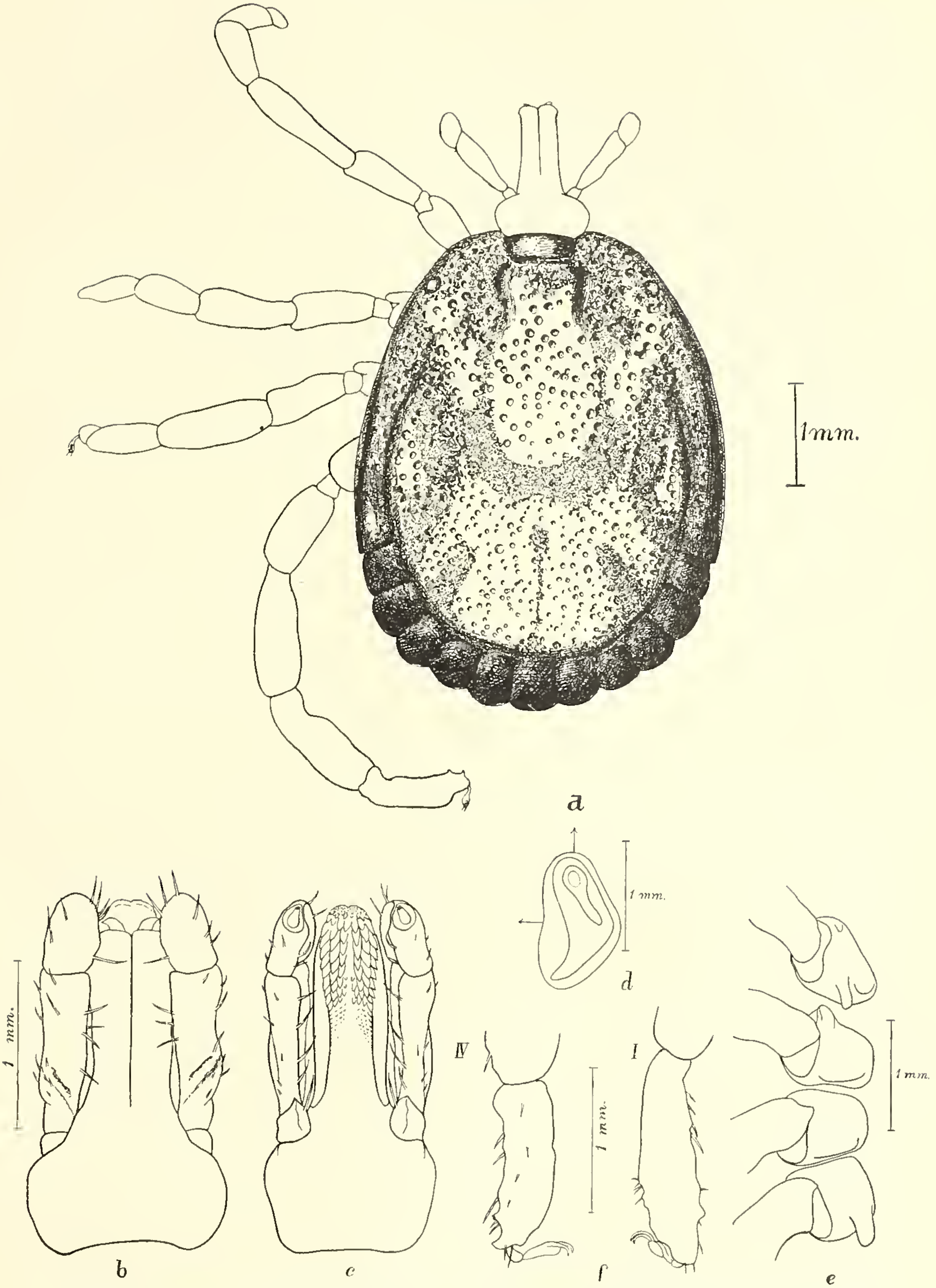

Fig. 2. *Amblyomma variegatum* var. *nocens* ♂: (*a*) dorsum; (*b*) capitulum, dorsal aspect; (*c*) capitulum, ventral aspect; (*d*) spiracle; (*e*) coxae I–IV; (*f*) tarsi I and IV. (C. 371. L.E.R. del.)

Tarsi abruptly attenuated; tarsi I unarmed, tarsi II, III and IV bicalcarate.

Female: undescribed. The donor of the specimens gave a short verbal description of the female of this variety, from which it may be presumed that it conforms very closely to the type.

Origin: Rhodesia, South Africa. Host not specified. Described from 2 ♂'s collected by Mr E. M. Jarvis, F.R.C.V.S., of the Dept. of Agriculture, Southern Rhodesia.

Mr Jarvis furnished the following notes:

This tick has become notorious among Rhodesian stock owners as the "Pyaemia tick." It is attributed to be the causative agent in the transmission of pyolymphangitis in equines; its bites are so severe as to cause violent inflammation and sloughing of the mammae in cows. It also attacks the heads of native children, causing sloughing of portions of the scalp.

Native districts: Umtali, Inyanga, Makoni, Melsetter, Portuguese Baruwe, Portuguese Mañica and the Zambesi Co.'s Territory. The tick is found chiefly in the bush veldt, at elevations of 2000 to 3000 feet, seldom above 4000 feet.

Amblyomma fiebrigi sp. n.

Figs. 3, *a–g*, and 4, *a–e*.

Male: body 3·4 × 2·37 mm., ovate, moderately elongate.

Scutum dull brown with indistinct pale spots (indicated in the figure by dotted outline); extreme margins and intervals of festoons darker brown; scapular angles almost pointed. Marginal groove absent. Cervical grooves in the form of short deep oval pits. Punctations numerous, rather small, regularly distributed. Eyes flat, large, indistinct. Festoons well defined, longer than broad. *Capitulum* (*l.* 1 mm.); basis capituli rectangular, with rounded lateral margins; twice as broad as long; postero-lateral angles barely salient. Palps short, clubbed; article 3 more than half as long as article 2. Hypostome spatulate, extremity rounded, emarginate, dentition 3|3. *Venter* wrinkled, glabrous. Genital orifice opposite coxae II. Extremities of circumanal groove prolonged anteriorly and fused with the genital groove on either side. Spiracles relatively large, broadly elongate comma-shaped. *Legs* short and stout. Coxae I with two short stout contiguous spurs of which the external is slightly the longer; coxae II, III and IV each armed with a short blunt spur, largest on coxae IV

Tarsi relatively small, abruptly attenuated; tarsus I slightly humped beyond the pseudo-articulation, unarmed; tarsi II, III and IV bicalcarate.

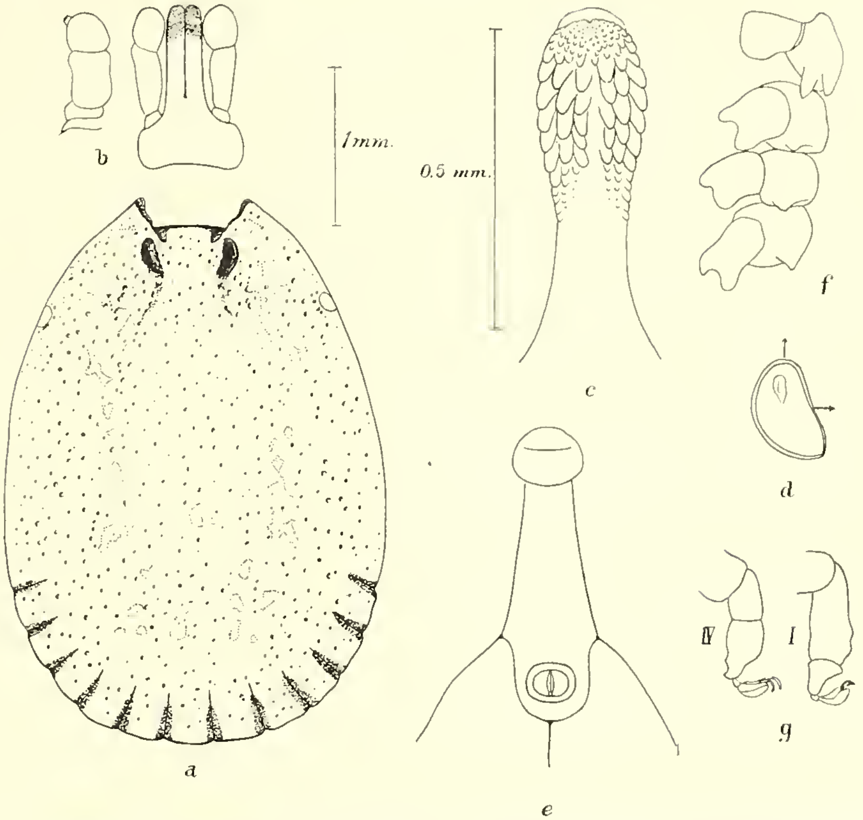

Fig. 3. *Amblyomma fiebrigi* ♂: (*a*) dorsum; (*b*) capitulum, dorsal and lateral aspects; (*c*) hypostome; (*d*) spiracle; (*e*) genital and anal grooves; (*f*) coxae I–IV; (*g*) tarsi I and IV. *b*, *d*, *e*, *f* and *g*—same magnification as *a*. (C. 6. L.E.R. del.)

Female (a slightly distorted specimen, semi-gorged): *body* 4·4 × 3·7 mm., dorsum almost black, glabrous, scattered with coarse punctations. Marginal groove present, fine and superficial, incomplete, commencing a little behind the lateral angles of the scutum and terminating at the antepenultimate festoon. Postero-median and accessory grooves shallow and ill defined. Festoons present, intervals fine and shallow. *Scutum* broader than long (2·15 × 2·44 mm.), almost rhomboidal; postero-lateral margins slightly convex and sinuous; colour as in ♂, with indistinct pale markings. Cervical grooves, scapular angles and punctations as in ♂. Eyes situated at the lateral angles of the scutum. *Capitulum* (*l.* 1·26 mm.); basis capituli more trapezoidal than in the ♂; porose areas almost circular, large and deep, the interval, about equal to the diameter of a porose area, is occupied by two consecutive median depressions. Hypostome similar to that of the ♂. *Venter* almost glabrous, a few very minute whitish hairs on the peripheral portion. Vulva opposite the interspace between coxae II and III. Genital grooves indistinct; circumanal and ano-median grooves deeply incised. Spiracle comma-shaped, with a rounded "tail." *Legs* similar to those of the ♂, spurs on coxae smaller.

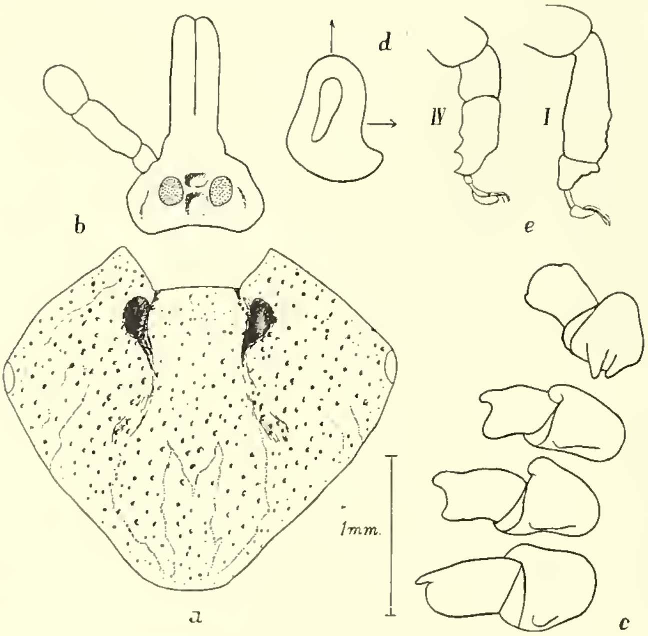

Fig. 4. *Amblyomma fiebrigi* ♀ : (*a*) scutum; (*b*) capitulum, dorsal aspect; (*c*) coxae I–IV; (*d*) spiracle; (*e*) tarsi I and IV. (C. 6. L.E.R. del.)

Origin: Described from 1 ♂ and 1 ♀, collected at San Bernardino, Paraguay, S. America, by Dr Karl Fiebrig. Host not specified.

Professor L. G. Neumann, of Toulouse, to whom the specimens were submitted, and who kindly gave his opinion concerning them, made the following comments: "se rapproche beaucoup de *A. göldii*. Les différences consistent dans les ponctuations, qui sont plus fortes dans *A. göldii*, et surtout dans l'hypostome qui chez ce dernier a 4|4 files de dents, tandis qu'il n'en a que 3|3 dans votre espèce. La forme de l'écusson de la ♀ est différente aussi dans les deux espèces."

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